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Mini Review

Recent progress in vaccine development targeting pre-clinical human toxoplasmosis

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Abstract

Toxoplasma gondii is an intracellular parasitic organism affecting all warm-blooded vertebrates. Due to the unavailability of commercialized human *T. gondii* vaccine, many studies have been reported investigating the protective efficacy of pre-clinical *T. gondii* vaccines expressing diverse antigens. Careful antigen selection and implementing multifarious immunization strategies could enhance protection against toxoplasmosis in animal models. Although none of the available vaccines could remove the tissue-dwelling parasites from the host organism, findings from these pre-clinical toxoplasmosis vaccine studies highlighted their developmental potential and provided insights into rational vaccine design. We herein explored the progress of *T. gondii* vaccine development using DNA, protein subunit, and virus-like particle vaccine platforms. Specifically, we summarized the findings from the pre-clinical toxoplasmosis vaccine studies involving *T. gondii* challenge infection in mice published in the past 5 years.

Keywords: *Toxoplasma gondii*, vaccine, virus-like particle, protein subunit, DNA

Introduction

Parasitic diseases are frequently neglected despite their importance and impact on human life. Toxoplasmosis, caused by the Apicomplexan parasite, *Toxoplasma gondii*, is a neglected disease of global importance. Global statistics demonstrate that *T. gondii* affects more than a third of the world's population [1], although seroprevalence can vary across regions [2]. These intracellular parasites are generally asymptomatic in healthy adults but can have fatal consequences in pregnant women and immunocompromised adults. For instance, transmitting *T. gondii* from mother to fetus can result in several congenital disabilities or stillbirths [3]. Administering drugs, such as pyrimethamine and sulfadiazine, can limit *T. gondii* infection in patients. However, these drugs are only effective against tachyzoites and cannot exert their full effect against the tissue-dwelling bradyzoites [4].

Furthermore, drug-resistant *T. gondii* strains continue to emerge globally, resulting in treatment failures. Although the underlying mechanisms remain largely elusive, mutation in the dihydropteroate synthase (*dhps*) gene could contribute to drug resistance against sulfonamides in *T. gondii* clinical isolates [5,6]. The clinical drug-resistant isolates, TgCTBr4 and TgCTBr17, acquired from newborn patients in Brazil, were reported to be less susceptible to pyrimethamine and sulfadiazine treatments [7].

Vaccines are highly desirable prophylaxis strategies that limit the dissemination of para-

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Conflict of interest
We declare that this research was conducted without any intention for commercial or financial benefits.

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sites. Efforts to develop an efficacious vaccine against toxoplasmosis have been ongoing for decades. To date, only one toxoplasmosis vaccine is commercially available. Toxovax is a live-attenuated *T. gondii* S48 strain that cannot be used in humans as safety profiles have not been clinically evaluated [8]. The exact reasons for prioritizing veterinary toxoplasmosis vaccine development over their clinical counterpart remains unknown, but congenital toxoplasmosis in ewes exhibited a considerable problem in the agricultural sector. Additionally, attaining regulatory approval is less stringent for veterinary vaccines than clinical ones [9].

Despite massive advances in vaccinology, an effective human vaccine for toxoplasmosis remains unavailable. After establishing toxoplasmosis as a significant foodborne infectious disease in the western hemisphere [10], it was not perceived as a threat to the general public. Clinical toxoplasmosis vaccine development has progressed rather slowly. Therefore, vaccine development remains a top priority. Herein, we briefly summarized some antigen components of *T. gondii* used in several vaccine platforms and highlighted advances in *T. gondii* vaccine development. We addressed several advantages and pitfalls of each platform that either promote or impede their development.

What is a vaccine and how do they work?

Vaccines are immune response-inducing biological products that confer protection against a specific infectious disease by exposing pathogenic agents to the host. The vaccine must express one or more antigens derived from the disease-causing pathogen [11]. The immunity induction mechanism is similar for most vaccines, irrespective of the target pathogen or platform. After entry of vaccine antigen, they are transported to compartmentalized secondary lymphoid organs, such as lymph nodes. The antigens activate B cells with specific receptors that recognize these foreign antigens. Once activated, the B cells present the processed vaccine antigen to the T cells and induce cellular signals that stimulate their proliferation and differentiation. The activated B cells produce short-lived plasma cells that secrete large quantities of antibodies. They induce germinal center responses, ensuring the production of memory B and long-lived plasma cells [12]. Simultaneously, antigen-presenting cells, such as dendritic cells, can cross-present the vaccine antigens to the T cells, signaling their differentiation into effector and memory T cells. Combined, these intricate processes contribute to the well-being of vaccinees by creating an immunological memory that confers rapid and robust protection against the target pathogen.

DNA vaccines

Molecular properties of DNA vaccines

Using nucleic acids for eliciting immune responses in hosts was first reported in the early 1990s by Tang et al. [13]. Since its discovery, DNA vaccines rapidly emerged into the scientific limelight and were actively researched. Structurally, DNA vaccines are composed of a bacteria-derived plasmid encoding a specific antigen of interest whose expression is controlled by a strong viral promoter for optimal gene expression in vivo, such as the cytomegalovirus (CMV) or the simian virus 40 (SV40). The precise mechanism underlying how

these DNA vaccines induce cellular and humoral immune responses despite their low expression levels in hosts remains largely unknown. However, 3 possible mechanisms describe how these vaccines may facilitate antigen presentation [14]. First, upon delivery into hosts by the parenteral route of immunization, the plasmid DNA encoding the antigen is internalized by somatic cells in the vicinity, such as myocytes or keratinocytes. The antigens are transcribed within these cells and eventually presented to $CD8^+$ T cells via the membrane histocompatibility complex (MHC) class I. Second, the antigen-presenting cells (APCs), such as dendritic cells, are recruited to the injection site. These cells become transfected by the plasmid DNA and present the expressed antigen of interest via MHC I and II. Last, the plasmid-infected somatocytes are phagocytosed by APCs to enable cross-priming and antigen presentation to $CD4^+$ and $CD8^+$ T cell subsets.

There are several factors favoring DNA vaccines over traditional vaccines. The production costs for DNA vaccines are relatively lower than traditional vaccines. Also, because infectious pathogens are not being introduced into the host, this vaccination approach is safe for use [15]. While DNA vaccines appear promising, there are safety concerns even if an infectious pathogen is not used for immunization. For instance, the possibility of antigen-encoding plasmid DNA integration into the host chromosome is one such consequence [16], though later studies revealed that the probability of genetic integration is extremely low [17]. Furthermore, DNA vaccines are weakly immunogenic. Specifically, suboptimal vaccine efficacies were reported from DNA vaccine studies conducted in non-human primates, as indicated by the low levels of antibody responses [15]. Based on these profiles, several DNA vaccines against various infectious diseases have undergone clinical evaluations but a clinical DNA vaccine trial for toxoplasmosis remains unreported.

Current progress in *T. gondii* DNA vaccine development

DNA vaccines are the most prevalent vaccine platforms being investigated throughout the world. The sheer amount of DNA vaccine-based publications skyrocketed in the early 2000s, and its popularity has remained unchanged [18]. This research trend is no exception for *T. gondii* vaccines, as most studies revolve around DNA vaccines. Despite the extensive research, most DNA vaccine results were suboptimal, while a few studies reported exceptional findings. Different *T. gondii* antigens conferred differing degrees of protection in mice. For example, DNA vaccines expressing the dense granule (GRA) 39 antigen prolonged the survival of Kunming mice by 20 days, but none could survive the challenge infection with the RH strain. Cyst burden reduction upon challenge infection with 10 cysts of PRU strain was suboptimal, as not even 50% cyst burden reduction was observed [19]. Contrastingly, GRA24-expressing DNA vaccines prolonged the survival of RH-infected BALB/c mice up to 30 days post-infection [20]. This was also the case with *T. gondii* Myc regulation 1 (MYR1)-expressing vaccine, which significantly prolonged the survival of immunized mice against RH challenge infection [21].

Several strategies improved the protective efficacy but were only marginally effective. Adjuvanting DNA vaccines had a minor effect on the vaccine's protective efficacy. Although supplementing the *T. gondii* GRA7 DNA vaccine with the calcium phosphate nanoparticle adjuvant prolonged the survival of immunized mice, it was only 2 days longer than the unadjuvanted control group [22]. Conflicting results were observed from multi-antigenic vac-

Table 1. Protective efficacy of DNA vaccines expressing various *T. gondii* antigens

Antigen	Mouse strain	Challenged *T. gondii* strain	Survival rate (duration)[a]	Reference
GRA24	Mouse (BALB/c)	Type I: RH	0% (32 days)	[20]
MYR1			0% (36 days)	[21]
MIC3, ROP9, SAG2			> 30%	[24]
MIC5, MIC16	Mouse (Kunming)	Type I: RH Type II: PRU	0% (26 days) ND[b]	[23]
GRA39			0% (20 days) ND[b]	[19]

[a]From challenge infection to all fatal.
[b]Not determined.

cines. Combined immunization with DNA vaccines expressing the microneme proteins (MIC) 5 and 16 as antigens reduced the brain cyst burden by half in mice challenged with the PRU strain but, as with other vaccines, failed to confer prolonged protection against the RH strain [23]. On the contrary, multi-antigenic DNA vaccines expressing the SAG2, rhoptry protein (ROP) 9, and MIC3 ensured that immunized mice survived the challenge infection with the highly virulent RH strain regardless of the infection doses [24]. Protection induced by these DNA vaccines was tabulated and briefly described (Table 1).

Protein subunit vaccines

Molecular properties of protein subunit vaccines

Protein subunit vaccines use a small fraction of a pathogenic agent's antigenic component to elicit immune responses in vaccinees. Like the traditional inactivated whole-organism vaccines, protein subunit vaccines are incapable of replicating in hosts and are safe but possess low immunogenicity. Therefore, protein subunit vaccines often require multiple immunization doses or adjuvant incorporation to achieve long-lasting immunity [25]. With the introduction of recombinant DNA technology and advancements in molecular biology, mass-producing foreign genes of interest in various expression systems has become feasible. Bacterial expression systems are frequently used to produce large quantities of protein of interest at a low cost. However, given the nature of prokaryotic organisms, proteins are misfolded, and post-translational modifications (PTMs) observed in mammals are lacking [26]. The need for downstream purification for endotoxin removal and processing of expressed antigens further hampers this. Like the bacterial expression system, yeast and insect cells can rapidly produce significant amounts of proteins of interest. While PTMs occur in these organisms, glycosylation patterns are not identical to those observed in mammalian cells [27]. Based on PTM, mammalian cells would be ideal for antigenic protein production. However, improvements are needed as mammalian cell-derived antigen yields are relatively lower than antigens produced in the aforementioned expression systems.

Current progress in *T. gondii* recombinant protein subunit vaccine development

Like DNA vaccines, much progress has been made using recombinant subunit vaccines. Subunit protein vaccines are safe, but their immunogenicity pales in comparison to other

Table 2. Efficacy of *T. gondii* vaccines based on protein subunit

Antigen	Mouse strain	Challenged *T. gondii* strain	Survival rate (duration)[a]	Reference
ASP3	Mouse (BALB/c)	Type I: RH	0% (18 days)	[29]
PRX1		Type II: PLK	< 70%	[30]
MIF, CDPK3, 14-3-3		Type I: RH Type II: PRU	90% ND[b]	[31]

[a]From challenge infection to all fatal.
[b]Not determined.

vaccine platforms [28]. While some protein-based *T. gondii* vaccines are protective, others failed to elicit desirable protection. The latter was predominantly observed in studies that utilized ubiquitous eukaryotic proteins as antigens. Vaccines expressing the *T. gondii* aspartic protease 3 (ASP3) prolonged the survival duration by 11 days against RH challenge infection [29]. *T. gondii* peroxiredoxin 1 (PRX1) vaccine failed to confer complete protection against the moderately virulent type II PLK strain [30]. However, as with DNA vaccines, conflicting protection results were observed from subunit vaccines (Table 2). Cocktail subunit vaccines conferred protection against types I and II *T. gondii* lineages. Intramuscular immunization with subunit proteins *T. gondii* macrophage migration inhibitory factor, calcium-dependent protein kinase 3, and the 14-3-3 protein resulted in complete protection against RH tachyzoite and PRU strains [31]. Given this circumstance, more research on improving these vaccines' protective efficacy is required.

Virus-like Particle (VLP) vaccines

Molecular properties of VLP vaccines

Although VLPs appear similar to protein subunit vaccines, they are not necessarily the same and should be categorized differently. VLPs are highly immunogenic self-assembled particles that mimic the structural aspects of native virions. However, these particles are inherently safe due to the lack of genetic material. Molecular and structural factors that contribute to the high immunogenicity of VLP-based vaccines have been described in detail [32], and as such, these aspects will be briefly described. In VLPs, antigens of interest are repetitively presented in a dense array which is critical to mounting efficient immune responses against the target antigen [33,34].

Furthermore, because the size of VLPs is less than 200 nm, they are rapidly trafficked into the lymph nodes [35]. The surface charge is another structural property of VLPs that improves immunogenicity compared to protein subunit vaccines. For example, particle-based vaccines possess charged surfaces that enhance their interaction with professional APCs, which may not be accurate for solubilized antigens [36]. Nonetheless, there are limitations to VLP vaccine technology, such as production costs. Like protein subunit vaccines, PTM must be considered during VLP vaccine assembly.

Current progress in *T. gondii* VLP vaccine development

To date, all VLP-based vaccine studies reported are chimeric, expressing parasitic antigens

Table 3. Protective efficacy of *T. gondii* vaccines based on virus-like particle

Antigen	Mouse strain	Challenged *T. gondii* strain	Survival rate (duration)[a]	Reference
MIC8	Mouse (BALB/c)	Type I: RH	100%	[37]
ROP13		Type II: ME49		[38]
IMC, ROP18, MIC8				[47]
ROP18, MIC8		Type I: GT1 Type II: ME49	0% (17 days) ND[b]	[42]
IMC, ROP18, MIC8		Type I: GT1	20%	[43]
B and T cell epitopes		Type I: RH Type II: ME49	0% (20 days) ND[b]	[45]

[a]From challenge infection to all fatal.
[b]Not determined.

on the surface of influenza virus matrix protein 1. Surprisingly, VLP immunization elicited considerable protection against virulent type I and moderately virulent type II strains in mice, such as those expressing MIC8 [37] and ROP13 [38] as surface antigens (Table 3). In a comparative study, VLPs expressing ROP18 antigens were more efficacious than those expressing ROP4 [39]. Similar to DNA and subunit vaccines, a multi-antigenic vaccine approach enhanced the protective efficacy of VLP vaccines. While ROP4 and ROP13 VLPs were protective and ensured 100% survival [40], VLPs co-expressing ROP4 and ROP13 antigens led to brain cyst burden reduction compared to VLPs expressing either antigen alone in BALB/c mice [41]. VLPs co-expressing MIC8 and ROP18 reduced the parasite burden following challenge infection with the *T. gondii* GT1 strain [42]. Further supplementing this vaccine with the inner membrane complex subcompartment protein 3 (IMC) conferred partial protection against the virulent GT1 strain but elicited complete protection against ME49 [43,44].

A research group demonstrated that chimeric hepatitis B virus-based VLPs expressing CD8 and CD4 T cell epitopes prolonged the survival of immunized mice upon challenge infection with *T. gondii* RH strain [45]. The impact of immunization regimen and adjuvant use was also evaluated. Herein, increasing the number of immunizations and supplementing adjuvants did not decrease brain cyst size but significantly reduced the cyst burden in ME49-infected mice [46,47]. More research on these *T. gondii* VLP vaccines is required, especially against the highly virulent strains, which are lethal even at small infection doses. However, the outlook for this vaccine platform appears promising. Furthermore, as all VLP-based vaccine studies were conducted in mice, evaluating their protective efficacy in higher-order eukaryotic organisms should be considered.

Conclusion

In summary, additional studies are required to improve the protective efficacy of *T. gondii* vaccines; however, the general outlook for their development seems promising. Numerous studies have proposed improving vaccine efficacy by optimizing immunization strategies, adjuvant usage, or identifying novel candidate antigens. Recent findings have demonstrated that pre-clinical toxoplasmosis vaccines can elicit cellular and humoral immune respons-

es in immunized mice, irrespective of the vaccine platform. Much of the *T. gondii* vaccine studies have focused on intermediate hosts, and vaccines targeting definitive hosts, including felines, are understudied. Furthermore, given the parasite's complex life cycle, antigens spanning multiple stages should be carefully evaluated based on their immunogenicity. Future investigations could attempt to address these shortcomings and employ novel strategies for vaccine development.

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References

1. Montoya JG, Liesenfeld O. Toxoplasmosis. *Lancet* 2004;363 (9425):1965-1976. https://doi.org/10.1016/S0140-6736(04)16412-X
2. Robert-Gangneux F, Dardé ML. Epidemiology of and diagnostic strategies for toxoplasmosis. *Clin Microbiol Rev* 2012; 25(2):264-296. https://doi.org/10.1128/cmr.05013-11
3. Jones JL, Lopez A, Wilson M, Schulkin J, Gibbs R. Congenital toxoplasmosis: a review. *Obstet Gynecol Surv* 2001;56(5):296-305. https://doi.org/10.1097/00006254-200105000-00025
4. Wang JL, Zhang NZ, Li TT, He JJ, Elsheikha HM, et al. Advances in the development of anti-*Toxoplasma gondii* vaccines: challenges, opportunities, and perspectives. *Trends Parasitol* 2019; 35(3):239-253. https://doi.org/10.1016/j.pt.2019.01.005
5. Silva LA, Reis-Cunha JL, Bartholomeu DC, Vítor RW. Genetic polymorphisms and phenotypic profiles of sulfadiazine-resistant and sensitive *Toxoplasma gondii* isolates obtained from newborns with congenital Toxoplasmosis in Minas Gerais, Brazil. *PLoS One* 2017;12(1):e0170689. https://doi.org/10.1371/journal.pone.0170689
6. Aspinall TV, Joynson DH, Guy E, Hyde JE, Sims PF. The molecular basis of sulfonamide resistance in *Toxoplasma gondii* and implications for the clinical management of toxoplasmosis. *J Infect Dis* 2002;185(11):1637-1643. https://doi.org/10.1086/340577
7. Silva LA, Fernandes MD, Machado AS, Reis-Cunha JL, Bartholomeu DC, et al. Efficacy of sulfadiazine and pyrimetamine for treatment of experimental toxoplasmosis with strains obtained from human cases of congenital disease in Brazil. *Exp Parasitol* 2019;202:7-14. https://doi.org/10.1016/j.exppara.2019.05.001
8. Dubey JP. Toxoplasmosis in sheep--the last 20 years. *Vet Parasitol* 2009;163(1-2):1-14. https://doi.org/10.1016/j.vetpar.2009.02.026.
9. Innes EA, Hamilton C, Garcia JL, Chryssafidis A, Smith D. A one health approach to vaccines against *Toxoplasma gondii*. *Food Waterborne Parasitol* 2019;15:e00053. https://doi.org/10.1016/j.fawpar.2019.e00053
10. Havelaar AH, Kirk MD, Torgerson PR, Gibb HJ, Hald T, et al. World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. *PLoS Med* 2015;12(12):e1001923. https://doi.org/10.1371/journal.pmed.1001923
11. Pollard AJ, Bijker EM. A guide to vaccinology: from basic principles to new developments. *Nat Rev Immunol* 2021;21(2):83-100. https://doi.org/10.1038/s41577-020-00479-7
12. Cyster JG, Allen CDC. B cell responses: cell interaction dynamics and decisions. *Cell* 2019;177(3):524-540. https://doi.org/10.1016/j.cell.2019.03.016
13. Tang DC, DeVit M, Johnston SA. Genetic immunization is a simple method for eliciting an immune response. *Nature* 1992; 356(6365):152-154. https://doi.org/10.1038/356152a0
14. Li L, Petrovsky N. Molecular mechanisms for enhanced DNA vaccine immunogenicity. *Expert Rev Vaccines* 2016;15(3):313-329. https://doi.org/10.1586/14760584.2016.1124762
15. Cui Z. DNA vaccine. *Adv Genet* 2005;54:257-289. https://doi.org/10.1016/s0065-2660(05)54011-2
16. Würtele H, Little KC, Chartrand P. Illegitimate DNA integration in mammalian cells. *Gene Ther* 2003;10(21):1791-1799. https://doi.org/10.1038/sj.gt.3302074
17. Faurez F, Dory D, Le Moigne V, Gravier R, Jestin A. Biosafety of DNA vaccines: new generation of DNA vectors and current knowledge on the fate of plasmids after injection. *Vaccine* 2010; 28(23):3888-3895. https://doi.org/10.1016/j.vaccine.2010.03.040
18. Lee J, Arun Kumar S, Jhan YY, Bishop CJ. Engineering DNA vaccines against infectious diseases. *Acta Biomater* 2018;80: 31-47. https://doi.org/10.1016/j.actbio.2018.08.033
19. Zhu Y, Xu Y, Hong L, Zhou C, Chen J. Immunization with a DNA vaccine encoding the *Toxoplasma gondii*'s GRA39 prolongs survival and reduce brain cyst formation in a murine model. *Front Microbiol* 2021;12:630682. https://doi.org/10.3389/fmicb.2021.630682
20. Zheng B, Lou D, Ding J, Zhuo X, Ding H, et al. GRA24-based

DNA vaccine prolongs survival in mice challenged with a virulent *Toxoplasma gondii* strain. *Front Immunol* 2019;10:418. https://doi.org/10.3389/fimmu.2019.00418
21. Zheng B, Ding J, Lou D, Tong Q, Zhuo X, et al. The virulence-related MYR1 protein of *Toxoplasma gondii* as a novel DNA vaccine against toxoplasmosis in mice. *Front Microbiol* 2019; 10:734. https://doi.org/10.3389/fmicb.2019.00734
22. Sun HC, Huang J, Fu Y, Hao LL, Liu X, et al. Enhancing immune responses to a DNA vaccine encoding *Toxoplasma gondii* GRA7 using calcium phosphate nanoparticles as an adjuvant. *Front Cell Infect Microbiol* 2021;11:787635. https://doi.org/10.3389/fcimb.2021.787635
23. Zhu YC, Ma LJ, Zhang JL, Liu JF, He Y, et al. Protective immunity induced by TgMIC5 and TgMIC16 DNA vaccines against toxoplasmosis. *Front Cell Infect Microbiol* 2021;11:686004. https://doi.org/10.3389/fcimb.2021.686004
24. Zhang D, Jiang N, Chen Q. Vaccination with recombinant adenoviruses expressing *Toxoplasma gondii* MIC3, ROP9, and SAG2 provide protective immunity against acute toxoplasmosis in mice. *Vaccine* 2019;37(8):1118-1125. https://doi.org/10.1016/j.vaccine.2018.12.044
25. Zepp F. Principles of vaccine design-Lessons from nature. *Vaccine* 2010;28(suppl):C14-C24. https://doi.org/10.1016/j.vaccine.2010.07.020
26. Rosano GL, Ceccarelli EA. Recombinant protein expression in *Escherichia coli*: advances and challenges. *Front Microbiol* 2014;5:172. https://doi.org/10.3389/fmicb.2014.00172.
27. Ducker C, Ratnam M, Shaw PE, Layfield R. Comparative analysis of protein expression systems and PTM landscape in the study of transcription factor ELK-1. *Protein Expr Purif* 2022; 203:106216. https://doi.org/10.1016/j.pep.2022.106216
28. D'Amico C, Fontana F, Cheng R, Santos HA. Development of vaccine formulations: past, present, and future. *Drug Deliv Transl Res* 2021;11(2):353-372. https://doi.org/10.1007/s13346-021-00924-7
29. 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 Trop* 2017;171:17-23. https://doi.org/10.1016/j.actatropica.2017.02.030
30. 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(4):e0176324. https://doi.org/10.1371/journal.pone.0176324
31. Liu F, Wu M, Wang J, Wen H, An R, et al. Protective effect against toxoplasmosis in BALB/c mice vaccinated with recombinant *Toxoplasma gondii* MIF, CDPK3, and 14-3-3 protein cocktail vaccine. *Front Immunol* 2021;12:755792. https://doi.org/10.3389/fimmu.2021.755792
32. Chu KB, Quan FS. Virus-like particle vaccines against respiratory viruses and protozoan parasites. *Curr Top Microbiol Immunol* 2021;433:77-106. https://doi.org/10.1007/82_2021_232
33. Bachmann MF, Rohrer UH, Kündig TM, Bürki K, Hengartner H, et al. The influence of antigen organization on B cell responsiveness. *Science* 1993;262(5138):1448-1451. https://doi.org/10.1126/science.8248784
34. Brune KD, Howarth M. New routes and opportunities for modular construction of particulate vaccines: stick, click, and glue. *Front Immunol* 2018;9:1432. https://doi.org/10.3389/fimmu.2018.01432
35. Manolova V, Flace A, Bauer M, Schwarz K, Saudan P, et al. Nanoparticles target distinct dendritic cell populations according to their size. *Eur J Immunol* 2008;38(5):1404-1 413. https://doi.org/10.1002/eji.200737984
36. Bachmann MF, Jennings GT. Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. *Nat Rev Immunol* 2010;10(11):787-796. https://doi.org/10.1038/nri2868
37. Lee SH, Kim AR, Lee DH, Rubino I, Choi HJ, et al. Protection induced by virus-like particles containing *Toxoplasma gondii* microneme protein 8 against highly virulent RH strain of *Toxoplasma gondii* infection. *PLoS One* 2017;12(4):e0175644. https://doi.org/10.1371/journal.pone.0175644
38. Kang HJ, Chu KB, Lee SH, Kim MJ, Park H, et al. Virus-like particle vaccine containing *Toxoplasma gondii* rhoptry protein 13 induces protection against *T. gondii* ME49 infection in mice. *Korean J Parasitol* 2019;57(5):543-547. https://doi.org/10.3347/kjp.2019.57.5.543
39. Kang HJ, Lee SH, Chu KB, Lee DH, Quan FS. Virus-like particles expressing *Toxoplasma gondii* rhoptry protein 18 induces better protection than rhoptry protein 4 against *T. gondii* infection. *Korean J Parasitol* 2018;56(5):429-435. https://doi.org/10.3347/kjp.2018.56.5.429
40. Kang HJ, Chu KB, Lee SH, Kim MJ, Park H, et al. *Toxoplasma gondii* virus-like particle vaccination alleviates inflammatory response in the brain upon *T. gondii* infection. *Parasite Immunol* 2020;42(6):e12716. https://doi.org/10.1111/pim.12716
41. Kang HJ, Lee SH, Kim MJ, Chu KB, Lee DH, et al. Influenza virus-like particles presenting both *Toxoplasma gondii* ROP4 and ROP13 enhance protection against *T. gondii* infection. *Pharmaceutics* 2019;11(7):342. https://doi.org/10.3390/pharmaceutics11070342
42. Lee SH, Kang HJ, Lee DH, Kang SM, Quan FS. Virus-like particle vaccines expressing *Toxoplasma gondii* rhoptry protein 18 and microneme protein 8 provide enhanced protection. *Vaccine* 2018;36(38):5692-5700. https://doi.org/10.1016/j.vaccine.2018.08.016
43. Lee SH, Kang HJ, Lee DH, Quan FS. Protective immunity induced by incorporating multiple antigenic proteins of *Toxoplasma gondii* into influenza virus-like particles. *Front Immunol* 2018;9:3073. https://doi.org/10.3389/fimmu.2018.03073
44. Lee SH, Chu KB, Kang HJ, Quan FS. Virus-like particles containing multiple antigenic proteins of *Toxoplasma gondii* induce memory T cell and B cell responses. *PLoS One* 2019;14(8): e0220865. https://doi.org/10.1371/journal.pone.0220865
45. Guo J, Zhou A, Sun X, Sha W, Ai K, et al. Immunogenicity of a virus-like-particle vaccine containing multiple antigenic epi-

topes of *Toxoplasma gondii* against acute and chronic toxoplasmosis in mice. *Front Immunol* 2019;10:592. https://doi.org/10.3389/fimmu.2019.00592
46. Kang HJ, Chu KB, Kim MJ, Lee SH, Park H, et al. Protective immunity induced by CpG ODN-adjuvanted virus-like particles containing *Toxoplasma gondii* proteins. *Parasite Immunol* 2021;43(1):e12799. https://doi.org/10.1111/pim.12799
47. Kang HJ, Chu KB, Kim MJ, Park H, Jin H, et al. Evaluation of CpG-ODN-Adjuvanted *Toxoplasma gondii* virus-like particle vaccine upon one, two, and three immunizations. *Pharmaceutics* 2020;12(10):989. https://doi.org/10.3390/pharmaceutics12100989