



Recent progress in vaccine development targeting pre-clinical human toxoplasmosis



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Abstract

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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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Conceptualization: Chu KB, Quan FS
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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 <i>T. gondii</i> 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	0% (26 days)	[23]
GRA39		Type II: PRU	ND ^b	
			0% (20 days)	[19]
			ND ^b	

^aFrom challenge infection to all fatal.

^bNot 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 <i>T. gondii</i> 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	90%	[31]
		Type II: PRU	ND ^b	

^aFrom challenge infection to all fatal.

^bNot 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 <i>T. gondii</i> 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	0% (17 days)	[42]
		Type II: ME49	ND ^b	
IMC, ROP18, MIC8		Type I: GT1	20%	[43]
B and T cell epitopes		Type I: RH	0% (20 days)	[45]
		Type II: ME49	ND ^b	

^aFrom challenge infection to all fatal.

^bNot 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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