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MINI REVIEW

Epitope-based vaccine as a universal vaccination strategy against *Toxoplasma gondii* infection: A mini-review

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ABSTRACT

Despite the significant progress in the recent efforts toward developing an effective vaccine against toxoplasmosis, the search for new protective vaccination strategy still remains a challenge and elusive goal because it becomes the appropriate way to prevent the disease. Various experimental approaches in the past few years showed that developing a potential vaccine against the disease can be achievable. The combination of multi-epitopes expressing different stages of the parasite life cycle has become an optimal strategy for acquiring a potent, safe, and effective vaccine. Epitope-based vaccines have gained attention as alternative vaccine candidates due to their ability of inducing protective immune responses. This mini-review highlights the current status and the prospects of *Toxoplasma gondii* vaccine development along with the application of epitope-based vaccine in the future parasite immunization as a novel under development and evaluation strategy.

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Introduction

Toxoplasmosis is a prevalent disease caused by *Toxoplasma gondii* (*T. gondii*), which is a zoonotic parasite that infects humans, domestic, and wild mammals [1,2]. It is a significant, life-threatening disease with medical, veterinary, and economic importance worldwide [3,4]. Immunocompetent individuals infected with toxoplasmosis are usually asymptomatic or might have mild symptoms, while, this disease in immunocompromised patients can be quite severe or even fatal [5,6]. Despite several available antiparasitic chemical drugs used to prevent or cure the infection and to limit and control the spread of *T. gondii* parasite in an infected host, these drugs still have limited efficacy and are not absolutely safe and could cause severe side effects [7–9]. Thus, acquiring safe and effective vaccine to control the vital impact of toxoplasmosis in both humans and animals is urgently needed [10].

Intensive efforts and significant advances toward acquiring an effective vaccine are under way to control infection and limit the incidence of the disease; however, no vaccine has, thus, far been available for use in humans [11–13]. Currently, the live attenuated tachyzoites of the strain S48 (commercially named "Toxovax") is the only approved vaccine for veterinary use. This vaccine was unfortunately shown limited efficacy [14].

Consequently, numerous studies on toxoplasmosis vaccination have been conducted and different forms of the parasite or parasitic antigens were tested, including inactivated or life attenuated vaccine, crude or recombinant antigen, subunit or multi-antigenic vaccines, and DNA

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vaccine [15]. Interestingly, the results indicated acquiring an effective vaccine against toxoplasmosis can be achieved.

The lack of available vaccine highlights the needs of exploring alternative reagents that can be used for immunization purposes. Further discussion was conducted to address the challenges, along with difficulties to acquire potential vaccine construct. Many suggestions were proposed and put forward in order to identify possible directions of the future research studies on the development of potential vaccines against *T. gondii*. In response to this scenario, the scientific suggestions were based on paying special focus on multi-epitope antigens that contain various immunoreactive epitopes of different *T. gondii* antigens. The potential use of the epitope-based antigen was explored to develop a new approach that expected to meet the demand of achievement of an effective vaccine. Characterization and identification of immunogenic epitopes from organism antigens could help in developing sensitive diagnostic assays, potential vaccines, as well as effective therapeutic agents [16].

The application of epitope-based vaccine in the immunization attempts of various infections has shown encouraging results and proved to stimulate protective cellular and humoral immunity [17–21]. Accordingly, epitope-based vaccine has suggested as a new potential candidates for acquiring a novel and effective *T. gondii* vaccine.

To date, the large number of immunization attempts highlight that future *T. gondii* vaccination approaches should apply antigens with potential to stimulate protective immunity against the parasite and are expressed in most of the parasite life stages [22,23]. Therefore, epitope-based vaccine features excellent immunogenicity and is valuable for acquiring new immunization strategy [22,24]. This review summarizes the approaches in developing vaccines against toxoplasmosis with emphasis on epitope-based vaccine and describes the designing and construction strategies of such vaccines, the advantages, disadvantages, and the current applications of these types of vaccines in toxoplasmosis vaccination strategies.

Approaches in Development of Vaccines Against Toxoplasmosis

The development of protective vaccines against *T. gondii* parasite can reduce the high incidence of the disease and prevent the clinical outcome in humans and animals [24,25]. Therefore, effective immunization is expected to reduce the shedding of the oocyst and prevent the cyst formation. Such vaccination would significantly reduce parasite transmission to intermediate hosts and definitely improve disease control [24]. Economically, the vaccine could also reduce losses in the livestock industry [26]. Thus, achieving effective vaccines against toxoplasmosis is a high priority and extremely important, given the high incidence of the disease worldwide, as well as the serious veterinary and clinical outcomes of the parasite, including chorioretinitis, abortions, mental defects, and death [26,27].

During the last 20 years, different immunization strategies in the achievement of effective *T. gondii* vaccine have been investigated. Consequently, the protection level has been evaluated with different types of immunogens, including the following: life-attenuated parasites, killed vaccines, native parasite antigens, recombinant antigens, and DNA vaccine; these immunogens have been tested as a new immunization strategy [22–25]. Moreover, inoculation of live parasites significantly induces effective immune protection against toxoplasmosis reinfection [28].

Despite the significant efforts in developing *T. gondii* vaccine, only the live attenuated tachyzoites of strain S48 (commercially named "Toxovax") was approved and licensed in 1992 to minimize the abortion rate in sheep. Unfortunately, this vaccine cannot be used for human immunization because of the risk of reverting to a virulent form; likewise, the vaccine may be pathogenic in immunocompromised patients [24]. Toxovax has a short shelf life and entails high costs [22]. In addition, immunization with other strains (ts-4) has been widely used in *T. gondii* immunization studies, providing significant resistance against cyst formation but partial protection against congenital toxoplasmosis. This approach increased the survival rate during acute toxoplasmosis [29]. Mouse inoculated with the temperature-sensitive mutant strain ts-4 induced protective immunity against lethal infection after a parasite challenge. By contrast, injection of mice with killed tachyzoite lysate provided no protection, neither alone nor with an adjuvant [21]. Despite the numerous vaccination strategies studied, as well as the vast knowledge of the molecular genetics, immunology, and pathology related to the *T. gondii* pathogen, no safe and protective vaccine exists for both humans and animals [22].

Thus far, all information obtained from the large number of immunization attempts have indicated that future *T. gondii* vaccination approaches should use antigens with the potential to stimulate cell-mediated immunity against the parasite, expressed in all parasite life stages and compatible with appropriate vaccination routes [22,23].

Vaccine in Cats and Livestock

The key step in controlling *T. gondii* infection is the prevention of oocyst formation in the definitive host (cats and other felines). Given that cats are the only source of oocysts, and that most probably, transmission of infection to intermediate hosts occurs through contaminated feces, the development of any protective vaccines to be used in

this species must be able to limit the shedding of oocyst to prevent environmental contamination by the oocysts [30]. Only, few studies have, thus, far focused on the cat vaccination. The use of a live mutant bradyzoite named T-263 was the first trial in which kittens were vaccinated [31]. After the oral inoculation, most of the kittens generated protective immunity; oocyst shedding was successfully prevented in 84% of the cats when challenged with the *T. gondii* parasite [31,32]. Unfortunately, T-263 has many disadvantages, including the need to use live bradyzoites and high costs [33]. Similarly, the effect of ^{60}Co-irradiated tachyzoites on the stimulation of protective immunity against the Beverley strains of *T. gondii* was investigated. The vaccine induced partial resistance after infection challenge; however, the vaccine showed disadvantages, such as the need for refrigeration and high costs [34]. Oocyst shedding was not reduced when DNA vaccines encoding rhoptry protein (ROP) 2 were used [35] even though DNA vaccine currently shows potential as an immunization tool.

In pigs, live *T. gondii* vaccines exhibited mild protection against the parasite but still showed risks of reverting to the virulent type and cause the disease [36]. Thus, in this species, the development of killed vaccines was necessary [22]. Recently, intradermal immunization of pigs with a DNA vaccine expressing GRA1–GRA7 of *Toxoplasma* antigens elicited high humoral and cellular immunity. The study proved that DNA vaccine could be effectively induce strong immune protection [37]. In addition, the potential of the tachyzoites of strain S48 in reducing the number of cysts in pork, and thus, improving food safety has been highlighted [38].

Recombinant and DNA Vaccines

Among the various approaches for acquiring effective *T. gondii* vaccines, the recombinant DNA technology is an alternative strategy of great potential [25,39]. The immunological effects of several *Toxoplasma* recombinant antigens have been widely evaluated in the last few years; these include surface antigens (SAGs), micronemal proteins, dense-granule proteins (GRAs), and ROPs [9,35,40,41]. Of them, only limited antigens were capable of inducing a strong and protective immunity. Unluckily, recombinant antigens tend to lack immunogenicity, especially vaccination trails of the intracellular pathogens. Therefore, uses of appropriate adjuvants are required to enhance their potency [42]. In addition, the production of the recombinant proteins within another expression system or organism could cause an allergic reaction [43].

The establishments of DNA vaccination strategies have opened a new perspective in the future of vaccine development [34,44]. Recently, various DNA vaccines against *T. gondii* have been developed and evaluated; some of them have shown promise [45]. The ability of such vaccines to induce high humoral and cell-mediated immunity makes it a promising vaccination strategy against intracellular pathogens including *Toxoplasma*[15]. DNA vaccines exhibit several advantageous such as easy to produce, easy to administer, stable, very immunogenic, and possess the potential for long-lasting immunity. In addition, DNA vaccines show high flexibility as several types of genes can be encoded in one DNA vaccine. Moreover, DNA vaccine has little risk of reverting to a virulent form or cause secondary infection [46].

Epitope-Based Vaccines

Antigenic variation and genetic polymorphisms represent major obstacles in the attempts of acquiring a successful vaccine of any particular pathogen. Therefore, understanding of these variation and polymorphisms in the populations is crucial for proper vaccine design and evaluation. Such data might also provide invaluable insights into parasite–host interaction [21,47]. The improved knowledge in bioinformatics tools, along with the advances in recombinant DNA technology, has allowed new strategies toward the design and production of novel epitope-based vaccines [48]. Epitopes or antigenic determinants are the minimal immunogenic part of any particular antigen, which are capable of inducing specific immune responses [49]. Accordingly, various immunogenic epitopes have been identified in different infectious pathogens and cancer, and they have significantly improved the development of potential epitope-based vaccines [50].

The improved understanding of how the immune cells recognize and interact with pathogenic antigens at the molecular and cellular level has significant contribution in the development and acquiring of rationally designed epitope vaccines [7]. The concept of epitope vaccines mainly relies on the prediction of immunodominant T and B cell epitopes that can elicit specific and protective immune response [51]. The antigenic variation in most infectious agents has impeded the development of effective vaccines [17]. Therefore, the use of immunogenic peptides in trials of acquiring epitope-based vaccine has recently drawn attention [51].

The most critical requirements include the proper identification of both T and B cells epitopes, as well as the selection of a novel and powerful approach to deliver those epitopes [48]. Immunization with multi-epitope vaccine expressing T-cell or/and B-cell epitopes against different pathogens showed significant increase in both cellular and humoral immunes responses, as well as prolonged survival time [30]. Numerous studies identified potential epitope-based antigens that could effectively induce high and protective immunity against diverse pathogens. The

approach has been used to develop and evaluate vaccines to various infectious agents, such as Influenza Virus [52], Human Immunodeficiency Virus [53], Epstein-Barr Virus [19], and hepatitis B virus [54].

Advantages and Disadvantages of Epitope-Based Vaccine

The potential advantages of using this vaccination strategy are as follows: it decreases the biohazard risk associated with other types of immunization; it has the ability to rationally engineer and optimize the epitope structure to increase potency in eliciting strong immunity; and it provides the opportunity to focus and generate specific immune responses to known conserved immunodominant epitopes [55]. In addition to the lack of infectious potential, epitope-based vaccine also shows chemical stability, and therefore, such kind of vaccines have been developed and tested against various infectious agents, including parasitic, bacterial, fungal, and viral infections, as well as cancers [51,56]. In the clinical trials of various cancers, peptide vaccine has entered phases I and II with satisfactory and promising clinical outcomes [57]. However, more effort is needed to eliminate the associated obstacles, including the necessity to have a better adjuvant, as well as the low or/and lack of resulting immunogenicity during antigen processing and presentation. Nonetheless, other study showed a significant progress in defying these limitations [51].

Identification of the Immunodominant Epitopes

Development of any potential epitope vaccine requires proper prediction and validation of highly immunogenic epitopes that are capable of inducing protective immune response and constitutes the basis of vaccines development as shown in Figure 1 [58]. In fact, significant barrier in designing such kind of vaccine is epitope identification [59]. Therefore, predicting or identifying T and B cell epitopes significantly furthered our understanding of how the immune response against the pathogens is generated and increased the chances of developing potential vaccines. The mechanism of action and how the epitope-based vaccines generate specific immune response were illustrated in Figure 2. Bioinformatic tools remain the vital option for analyzing immunogenic epitopes with high antigenicity and immunogenicity even though the inherent complexity of microbial antigens recognition complicates the process of epitope prediction [21,60]. Yet, significant efforts have been put toward acquiring novel strategies and efficient tools for epitope analysis. Consequently, different algorithms have been developed and tested for predicting and screening of possible epitopes, and the results indicate a promising strategy for vaccine development [59].

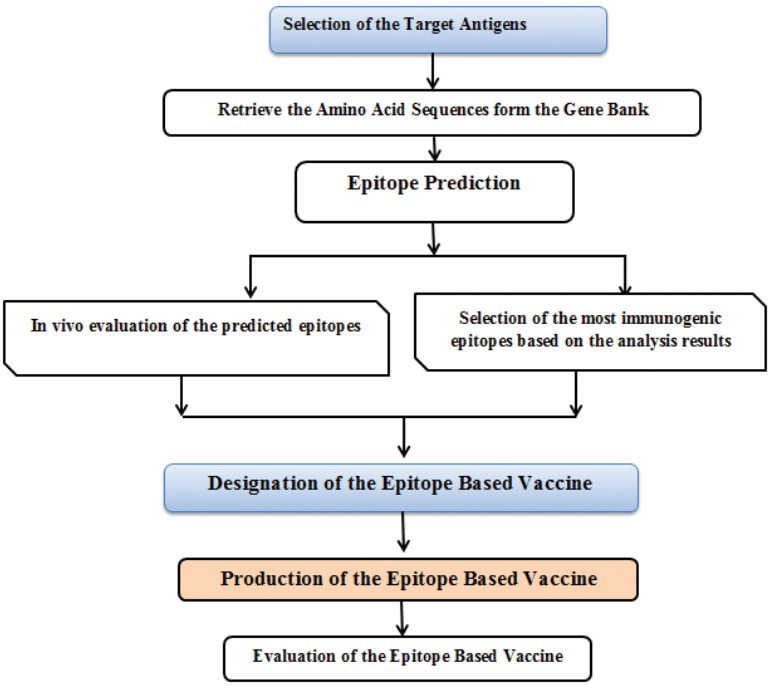

Figure 1. Schematic illustration of the epitope-based vaccine destination and construction.

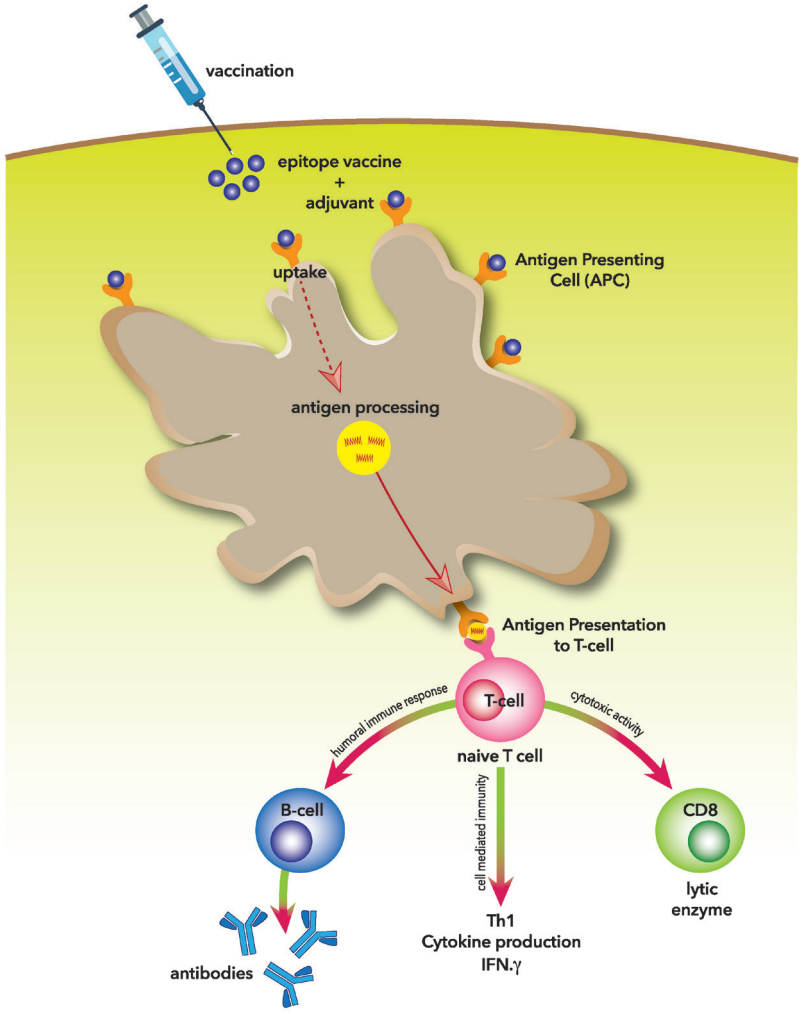

Figure 2. Mechanism of action of epitope-based vaccination.

Application of bioinformatics approaches in the analysis of conserved sequences and predicting of potential epitopes have been widely used against various pathogens. It represents a powerful alternative strategy of epitopes discovery that significantly reduces the cost, time, as well as the effort involved in the experimental approach of epitope screening. However, the variation among epitopic regions might affect the prediction process, and thus acquiring functional protective epitopes. Selection of immunogenic epitopes is crucial in designating any particular epitope-based vaccine; therefore, the differences in pathogens genotypes and subtypes should be taken into account.

Epitope-Based Vaccine Against Toxoplasmosis

Since the application of bioinformatics tools in the production of epitope-based antigen has become potential strategies to acquire a novel and powerful vaccine against infectious agents [61], considerable efforts have been made toward developing a promising epitope-based vaccine against toxoplasmosis (Table 1) [62,63]. Researcher assumes that construction of single- or multi-epitope-based antigen expressing potential B or/and T cell epitopes of both tachyzoite and bradyzoite specific antigens would greatly improve *T. gondii* immunization strategies [64]. Accordingly, several studies have been conducted and resulted in the identification of various promising epitopes that are capable of inducing protective immune response, and would possibly contribute to the attempts of developing a protective vaccine against *T. gondii* [7]. This evidenced by the significant immune protection generated in mice models [65,66].

Recently, a synthetic vaccine expressing nine epitopes predicted from GRA2, GRA7, and SAG1 of *T. gondii* was tested

Table 1. List of predicted Epitopes evaluated as potential Epitope-based vaccine.

Epitope	Antigen Gene	Reference
LGPVKLSAEGPT, TAAKTHTVRGFKV, SYFAADRLVP	SAG1, GRA2, GRA7	[41]
KLFETTDMY, VRQEAIARALARAAA	Anopheles mosquito salivary proteins	[70]
GNIEGQWALKNHSLVSLSEQVLVSCDNIDD	CPA (Cysteine peptidase A)	[59]
YSNIGVCK		[71]
QTLIAIHTLAIRYAN	*Paracoccidioides brasiliensis* gp43 antigen	[72]
RPPIFIRRL, sSVRDRLARL	EBNA3	[19]
Residues 137–160 and 197–211	VP1 gene of foot-and-mouth disease virus	[73]
(TAKDGMEYYNKMGELYKQ, (RCLLGFKEVGGKCVPASI)	Plasmodium knowlesi merozoite surface protein-142	[7]
TCPDKKSTA	SAG1 (59–67)	[9]
KSFKDILPK, STFWPCLLR, AVVSLLRLLK, SSAYVFSVK, AMLTAFFLR)	SAG1, SAG2, GRA5, SRS52A, GRA6	[17]

in BALB/c mice. Immunization with this multi-epitope vaccine significantly generates mixed Th1/Th2 antibody response and high production of IFN-γ cytokine [67]. Similarly, significant increase in the cellular and humoral immunity was generated when the mice was immunized with a multi-epitope vaccine containing two T cell epitopes and one B cell epitope of SAG1, GRA1, and GRA4. In addition, vaccinated mice obtained long-term survival rates compared with the unvaccinated controls [12].

In contrast, epitope vaccine composed of a single B or T cell epitopes has been used previously and confirmed to elicit strong immune responses, for instance, synthetic B and T cell epitopes identified from GRA2 antigen were able to stimulate both cellular and humoral immunity and to increase the survival rate of immunized animals [64]. Similarly, mice immunized with epitopes vaccine identified form ROP19 protein induced significant T and B cell immune response and also indicated effective protection following parasite challenge with PRU strain *T. gondii* cysts [68]. However, effective systemic and mucosal immunity was enhanced with both single and mixed peptides with a strong lymphoproliferative response associated with significant IFN-γ, IL-2, and IL-4 production, and a high level of specific antibody responses. In addition, partial immune protection against acute and chronic toxoplasmosis was also generated [7]. Furthermore, a combination of DNA/peptide vaccine significantly reduced the formation of the brain cyst among the immunized mice [69].

This emphasized the involvement of single or mixture of epitopes has shown to remarkably induce effective humoral and cellular immune response against toxoplasmosis. This could be powerful and efficient strategy that can be considered in the production of possibly protective vaccine candidate against toxoplasmosis.

Conclusion

The development of potential vaccine against *T. gondii* has significantly progressed in the last few years. Numerous experimental studies of preventive immunization have explored various forms of *T. gondii* antigens including live-attenuated vaccines, subunit vaccines, recombinant vaccine, and DNA vaccines [13]. Accordingly, significant strides have been conducted in antigen isolation and characterization, gene cloning, and immunological techniques. In addition to all the prevention strategies, new options to produce effective vaccines are currently needed as the appropriate way to prevent the disease [15].

Previous studies on developing effective vaccines against *T. gondii* revealed that vaccines that express only single antigen or single stage induce partial immune protection against the parasite [64]. Thus, a vaccine that expresses multiple stages of the parasite life cycle must be synthesized. Adopting bioinformatics to identify antigenic epitopes and theoretically arranging multiple epitopes in a single antigen could aid in the achievement of potential *T. gondii* vaccines. The use of epitope-based antigens is highly promising in the development of potential vaccine candidates that would generate lasting protective immune reaction against *T. gondii.* Furthermore, the use of epitope-based antigens could be an important approach in investigating the improvement of the disease vaccination in the future. Future studies should also consider the exploration of

appropriate adjuvants that can be used along with epitope-based vaccination strategies and establishing optimal immunization protocols along with evaluation criteria.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' contribution

All authors were equally contributed in the writing and approving this mini-review.

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