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Review Article

Vaccine Design Based on Live Attenuated Cells of *Toxoplasma gondii*: A Review

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Abstract

Toxoplasma gondii (*T. gondii*) is a well-known, commonly found pathogen by high proliferative activity in nucleated cells. This parasite has infected nearly one-third of the world's population. Infection can result in chorioretinitis in immunocompetent hosts, encephalitis in HIV/AIDS positive patients and spontaneous abortion or neonatal death in fetuses infected with congenital toxoplasmosis. Development of vaccines for toxoplasmosis is of major importance for reducing routes of disease transmission among various host species in the world. Live attenuated vaccines resemble natural infectious agents very closely. Therefore, these vaccines are good enhancers of the immune system, often imparting lifelong immunity with only one or two doses. In this study, we review the current status of live attenuated vaccine development for *Toxoplasma* infection.

Keywords: Bradyzoite, Live attenuated vaccines, Tachyzoite, *Toxoplasma gondii*

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1. Introduction

Toxoplasma gondii is recognized as a important phatogenic parasite, due to its high infective potential as well as its capacity to infect most nucleated cells in a large part of mammalian species, including livestock [1]. which parasite was prime discovered from organelles of the North African common gundi (*Ctenodactylus gondii*), a rodent, about a 100 years ago, following which cats were identified as the definitive hosts [2]. There are three separate pathogenic shapes; sporozoite (in oocyst), tachyzoite and bradyzoite (in tissue cyst). Intermediate hosts may be infected via meat infected

by tissue cysts, via food or water contaminated with parasite oocysts or via vertical transmission (trans placental) [3]. Infection can result in chorioretinitis in immunocompetent hosts, encephalitis in HIV/AIDS positive patients and miscarriage and neonatal death of the fetus due to congenital toxoplasmosis in pregnant women infected for the first time [4]. Treatment of this infection remains problematic as many drugs may produce intense side effects, in addition to the possibility that a relapse may occur at any time [5]. Production of an effective vaccine may prevent toxoplasmosis in humans and animals, leading to a decrease in the health expenditure of many countries and an improvement in the quality of life in infected patients [6, 7]. Vaccination against toxoplasmosis may be influential in decreasing infection transmission routes among various host species [7]. Only one commercial vaccine (Toxovax®) consisting of a live attenuated *T. gondii* S48 strain, has received approval for use against congenital infection [8]. However, this vaccine is costly, has a short shelf-life, and is known to cause adverse effects. Perhaps this vaccine constitutes a pathogenic form and is not fit for human utilization [9]. Several types of vaccines capable of activating the immune system of animals against *toxoplasma gondii* have been developed. These include inactivated and attenuated vaccines, Genetic engineering vaccines , subunit vaccines as well as RNA (as mRNA) and DNA (as plasmids) vaccines. However, only few of these have been approved for public utilization, due to a lack of efficiency or safety concerns [9-13]. Attenuated vaccines are produced using a live parasite type, which has been weakened in the lab and therefore is unable to cause disease. Such weakening of an organism is termed attenuation. Since attenuated vaccines very closely resemble a natural infection, they are good activators of the immune system and often create lifelong immunity with only one or two doses [14]. Although, there have been many attempts at producing prosperous vaccines against human parasites, no vaccine has been approved for use against toxoplasmosis in humans so far. Therefore, it may be concluded that a need exists for further research into the possibility of developing a suitable vaccine against toxoplasmosis.

2. Protective Immune Mechanism Against *T. gondii*

The initial step in toxoplasmosis infection is an innate immune response. The parasite immediately stimulates macrophages, which release the proinflammatory cytokines (IFN-γ and TNF-α) [15]. IFN-γ inhibits parasite propagation in the primary phase of tachyzoite infection, promotes an adaptive immune response, and stimulates T helper (*Th1*) cells to produce proinflammatory factors [16]. IFN-γ and TNF-α act cooperatively in the suppressing of tachyzoites by macrophages, and these two mediators induce an increase in the production of nitric oxide (NO) and free radicals, that may also effect parasite suppression [17]. While CD8+ T cells are highly capable of eradicating infected cells [18], they may cause anti-parasite effects with increasing levels of IFN-γ in macrophage cells [17]. Protection against *Toxoplasma* infection induces a cascade type activation of the immune system, mainly via IL-12, and next via CD4+ and CD8+ to develop protective immunity [16, 19]. Studies on CD4+ and CD8+ activity in humans

have shown that Tcells are cytotoxic to parasite-infected cells, which, in turn, may prevent tachyzoite growth in cells [20]. T helper (CD4+) induces apoptosis in toxoplasma infected cells by producing IFN-γ. Cytotoxic T cells attack and kill cells infected by T. gondii [21].

Th1 immune response activates the development of cellular immune response , this is necessary in order that eradicating *Toxoplasma* infection in cells and resisting tachyzoite replication in nucleus cells. Therefore, the production of inflammatory cytokines, as IFN-γ and TNF-α, seems to be responsible in order that the changing of tachyzoites into bradyzoite forms as well as for cyst formation in various tissues such as those found in brain tissue [22]. At this stage, the parasite becomes latent in individuals with strong immune systems, but in immunosuppressed patients, parasite cyst formation can cause cerebral toxoplasmosis as a result to cyst activation in addition to a metamorphosis switch [23]. The role of Th1 cells is vital for the progression of the protective response to both acute and chronic toxoplasmosis. Vaccination may stimulate IFN-γ making via T helper cells, and the resulting cytotoxic T lymphocyte cell (CD8+) responses to the parasitic infection may activate cellular immunity. Th2 activates CD4+ and B cells, both of which are responsible for the production of antibodies that suppress *Toxoplasma* infection [24, 25]. Increasing levels of IL-8 correspond to early acute inflammation against toxoplasmosis. IL-8 is one of the most important responsible cytokines for activating and recirculating neutrophils toward phagocytizes, for the purpose of killing or inhibiting *Toxoplasma* tachyzoites. Human intestinal epithelial cells infected by *Toxoplasma gondii* evoke quick secretion of IL-8 [26]. In patients with high levels of antibodies of acute toxoplasmosis(IgM+), serum levels of proinflammatory cytokines such as IL8 appear to be higher [27].

3. Strategy and Goals for Producing Vaccine Against *T. gondii*

Based on a review of current literature in regard to issues such as the designation of suitable vaccines against *Toxoplasma gondii also* the importance of routes of vaccine transfer, the goals of vaccination may include: 1) effecting a decline in oocyst excretion by cats in order to decrease environmental contamination; 2) limiting tissue cysts and acute parasitemia to protect against congenital toxoplasmosis in human and animals.

4. Vaccination Using Live Attenuated Parasites

T. gondii was first isolated in 1941, from the brain of a person fatally infected with congenital toxoplasmosis [28]. which strain, named RH, showed high virulent potency in laboratory animals, especially mice. The strain was proved to be secure in pig models and was undetectable in the tissues three weeks after the posted vaccination [29]. Immunization of guinea pigs using inactivated whole *T. gondii* tachyzoites of RH strain indicated several types of protection against a challenge [30]. The survival rate of mice, vaccinated with formalin fixed whole tachyzoites, was significantly increased following a challenge by *T. gondii* tachyzoites [31]. A tissue cyst called S48 was isolated from an

aborted bovine fetus in 1988. After vast passages (x 3000) in the laboratory, S48 was converted into an incomplete and attenuated strain [32]. Vaccination of sheep with S48 led to a decrease in abortions, a lowering of neonatal mortality and an increase in birth weight [11]. This strain, which produces IFN-γ via T cells (CD4+ and CD8+) is able to protect against toxoplasmosis [33]. Use of the above strain in pregnant *Aotus* monkeys was not successful, even though the strain proved to be safe for immunocompetent primates [34]. In goats, vaccination with strain S48 was found to be moderately effective [35].

The Environmental Protection Agency (EPA) indicated that oocysts may be a major environmental polluting agent, as well as the primary source of origin for infections in animals and humans [36]. Human outbreaks of toxoplasmosis have been attributed to oocyst pollution of water supplies [37]. Studies have indicated that oocyst shedding was prevented in 80% of young cats vaccinated with T-263 bradyzoites, when challenged with an oocyst-forming strain of *T. gondii*. Vaccination solely with live tachyzoites did not induce these effects [38]. In a study reporting improved efficacy of the vaccine, two oral doses of intact tissue cysts and released bradyzoites were administered to a group of cats. Oocyst shedding was not detected in the cats vaccinated with T-263, even forty-seven days later [38]. Immunization against Toxoplasmosis was induced by attenuated vaccine of *Toxoplasma* in Swiss Webster mice who had *mic1* and *mic3* deleted from their genome. With this vaccine, a reduction of more than 96% in brain cysts, also a decrease in penetration to fetuses was saw after a challenge. No sterile immunity was obtained despite these hopeful results [39]. However, development of the T-263 mutant strain from the brains of infected mice was found to be too cumbersome a process for large scale production, although it has been proven to be a good subject in vivo [14].

One of the most common vaccines that have been successfully employed for helminths and protozoa uses gamma irradiated strains [40]. Dosages of less than 1000 Gy with gamma source led to tachyzoites which could invade cells, but not able to grow and reproduce . but They were able to stimulate cellular immunity and slightly protection [41]. To reduce most of the biological alterations in *T. gondii* tachyzoites, leading to low efficacy of the vaccine, the dose of exposure was adjusted to 255 Gy with Cobalt-60. However, this radiation dose caused inactivation of the tachyzoite strain RH which could not induce immunity equal to that acquired from a natural infection [42]. A high level of immune protection has been achieved in mice immunized with irradiated ME49 strain cysts [43]. Similar studies have been conducted to decrease tissue cyst formation in immunized rats, pigs, and lambs with a low virulent ME49 strain [44-46]. Studies have indicated that pidotimod as an immunostimulant in combination with UV- attenuated *Toxoplasma gondii* vaccine displayed a synergistic role towards improve proper immune activity which may aid in improving efficacy against infection in mouse models [47]. Immunization with inactivated tachyzoites, independent of the adjuvant used, gave lower protection compared to live attenuated parasites [24]. Live attenuated vaccines gave better protective responses compared to subunit vaccines. Pigs vaccinated with a subunit vaccine based on crude rhoptry proteins, showed weak immunity against of tissue cyst formation [45]. A recent study

reported a novel and efficient live attenuated vaccine obtained from strain cps1-1, which showed good protection against type I and type II strains of *T. gondii* in mice. Use of irradiated tachyzoites or type I strain TS-4 as live attenuated vaccines, had the greatest success in imparting complete protection against killing type I challenges [12]. These researches as well as indicated a noticeable decrease in type II cyst loading compared to component vaccines or whole-dead parasite vaccines [48, 49] However, in immunocompromised hosts, vaccines based on live parasites like strain TS-4 could replicate and retain its potential for infection. Despite providing protection against parasite TS-4 strain, immunization does not constant and decreases significantly within months [12, 50]. Therefore, the value of such strains as candidates for an effective vaccine in humans and animals may be limited.

5. Live Attenuated Vectors

Completion of the human genome project and development of high throughput techniques in molecular genetics, combined with increasing knowledge of *T. gondii*, enabled critical details in the designation of new attenuated vaccines to be elucidated. These targeted methods have been used in order to produce parasites with decrease productive capacity and reduced virulence. Mutant parasites have been produced by either omitting an inalterable gene or inhibiting an essential gene expression [51]. The first vector was reported Recombinant *Mycobacterium bovis* BCG expressing GRA1 used in *T. gondii*. Which plan enabled the induction of a GRA1- particular cellular immune answer in sheep, however was not successful in outbred mice [24]. RH strain with deletion of carbamoyl phosphate synthetase II (CPSII) was a genetically engineered mutant to be extensively studied in related research [52]. Injected BALB/c mice were not killed by CPSII knock-out parasites, and infected mice stood against a deadly challenge by 200 pfu of *T.gondii* tachyzoites within 40 days [53]. Destruction of BAG1 only reduce the number of tissue cysts in mouse brains by roughly five-fold. Attenuated *Salmonella* strains have been assessed for efficacy as live vectors to deliver plasmid DNA by oral immunization [58]. The BALB/c mice vaccinated by a fusion protein of SAG1-SAG2 linked to cholera toxin A2/B, and produced in attenuated *Salmonella typhimurium* strain enhancement survival rate in mice immunized with BRD509/pSAG$_{1-2}$–CTA$_2$/B [59]. A improved vaccinia ankara vector expressing ROP2 displayed increased survival time following a fatal challenge by *T. gondii* tachyzoites [24]. GRA4 vaccine, made from recombinant plasmid and attenuated vaccine virus, prevented the formation of tissue cysts in BALB/c mice [60]. Survival rate of ICR mice vaccinated with attenuated Salmonella typhimurium strain linked to SAG1, DNA vaccine, and challenged with injected doses of 10^7 and 10^8 cfu tachyzoites form of T. gondii RH strain, increased from 10% to 20%, while the mortality rate in unvaccinated mice was perfect [61]. 100% survival and significantly reduced brain tissue parasite load was observed in mice vaccinated with GRA4 antigen delivered via recombinant attenuated Vaccinia virus, and challenged with infection by type II PLK/GFP tachyzoites [59].

Mic1-3KO was indicated as a proper vaccine candidate in mice after oral cyst challenge against congenital and chronic infection [62]. Using *T. gondii* SAG1 gene in adenoviral vector can stimulate effective immunity relying on Myeloid Differentiation Factor 88 and secreat pro-inflammatory cytokines [63]. Zhang et al., performed a study on *Neospora caninum*-based live attenuated vaccine vectors , expressing *Toxoplasma gondii* (TgSAG1). Mice immunized with these vaccines showed stimulation of responsive cellular immunity and increased viability when challenged with Beverley strain of T. gondii [64].

6. Reducing Oocyst Shedding By Vaccine Design in the Definitive Host

Cats are the lonely final hosts, which actively release *T. gondii* oocysts via feces into the environment and thereby contribute to an increase in *Toxoplasma* infection in intermediate hosts such as humans [65, 66]. Although, a few studies focusing on the vaccine designation on cats against *T. gondii* infections have been reported, these studies claim that oocysts shedding by cats may be stopped by immunization [67]. A live vaccine consisting of the mutant T-263 strain was used in cats. After oral inoculation with T-263 bradyzoites, 84% of vaccinated cats did not shed oocysts during the challenge [68]. which conservation was enhanced via treatmeant two doses of the live attenuated vaccine [38]. This vaccine was able to stop oocyst shedding to some extent when challenged with cysts of Beverley strain, but had the disadvantages of being expensive and requiring refrigeration. Studies showed that the T-263 vaccine can decrease seroprevalence toxoplasmosis in pigs and cats that living on the farms contaminated with oocytes [14]. Following a challenge with 3 different *T. gondii* strains, eight out of nine cats inoculated with tissue cysts of *T. gondii* strain ME-49 were immunized against oocyst excretion , showing that this strain was very capable of inducing cross protective immunity [69]. Most studies involving vaccination with live vaccine products against *T. gondii* in cats may induce appropriate protective cell-mediated immune responses, but also carry disadvantages in terms of safety, short shelf life and large-scale production.

7. Vaccine Studies in Food-Producing Animals

In Scotland and North America, *T. gondii* is the one of the major factors causing abortion in herds of sheep and goats [70]. In the United States, half of *Toxoplasma gondii* infection can be attributed to foodborne transmission [71]. The incidence of infection in Europe was 60% [72]. Meanwhile, meat products may play an important role in the transmission of infection to humans. As a result, meat products associated with sheep, goats, pigs and wide-range of poultry are suspected of being responsible for infections in humans [37]. Vaccination of animals via food to inhibit tissue cyst formation may be highly effective in decreasing transmission. In addition, utilization of cook insufficiently or raw meat products originating from infected animals may cause disease transmission especially in immunodeficient individuals [73].

One commercially available vaccine against *T. gondii* consists of live attenuated tachyzoites of strain S48 [8]. The potential of the S48 Toxovax® for reducing the development of tissue cysts in vaccinated sheep has been mentioned before. The RH strain has been used to immunize pigs in order to prevent the expansion of parasite cysts into other similar tissues [14]. Intramuscular vaccination (IV) with CpG-adjuvanted RH tachyzoites protected 52% of challenged pigs and provided effective immunity opposed to tissue cyst formation [74]. A new study indicates that, 100% of mice which had received porcine tissues from pigs vaccinated with S48 survived [75]. Only a limited number of studies have been performed to analyze the effects of immunizing food-producing animals with *T. gondii* vaccination. These studies were not able to demonstrate the protective efficacy of the vaccine clinically.

8. Conclusion

Toxoplasma is known as the most successful parasitic organism, due to its high potential to infect humans and a wide range of animals. A complete understanding of the lifecycle and transmission routes of this parasite, as well as its disease pathogenesis within different hosts has allowed us to implement the controls and interventions needed to produce a suitable vaccine against this parasite. The option of producing a vaccine that is effective in controlling and decreasing the parasite both in the hosts and the environment is important, but due consideration must be given to the fact that, live attenuated vaccines may cause protective immune responses in most animals. On the other hand, using such a vaccine in animals may prevent abortion during the primary stages of pregnancy. Several live attenuated parasites vaccines mentioned above caused protective responses similar to those caused by a natural infection without inducing pathogenesis in the body. However, Ovilis®Toxovax, the only vaccine that was commercialized for veterinary use, and readied for the market in Europe in 1988, in order to control miscarriages and stimulate a decrease in cyst formation in meat. This vaccine appears to decrease infection in ruminates as sheep, that are continuously exposed to oocyst contamination during wide-range grazing.

Many potent vaccines are available for immunization against viral and bacterial illnesses, but only a few vaccines have been developed successfully for veterinary protozoan illnesses and at present none of them are available for human use. It is suggested that further studies on *T. gondii* vaccines should focus on immunization methods and vaccine delivery strategies using antigens capable of triggering a protective response. In designing an effective attenuated vaccine, attention must be paid to several factors including induction of both humoral and cellular immune response, and a guaranteed standard of safety comparable to that of killed vaccines.

Issues related to live vaccines are mainly associated with safety and short shelf-life. Production of a vaccine which is capable of differentiating between vaccinated and infected animals may be ideal. Therefore, the use of knockout strains and virus vectors may be a desirable strategy to expand the use of vaccines against this parasite. Application of novel reverse genetic techniques on *Toxoplasma* that may allow the deletion of specific genes in order to inhibit its capability of reverting to virulence, as well as

the incorporation of mutant attenuated strains may be useful in the development of new vaccines.

At present many successful veterinary vaccines use live attenuated strains of a parasite because such vaccines closely resemble natural infections, with a reduced risk of causing disease or a prolonged and persistent infection as a reaction to the vaccine. Future strategies for the development of vaccines could be rely upon the use of attenuated live parasites in combination with manipulation via genetic engineering or on incorporation of subunits in attenuated strains.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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