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

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Abstract

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

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

Background

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

Toxoplasmosis in farm animals

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

Toxoplasmosis in laboratory animals

Experimental animals can be divided into two groups according to their susceptibility to *T. gondii* infection, rats and Old World monkeys are categorized in a resistant group, whilst mice, hamsters, guinea pigs and New World monkeys in the susceptible group. The variable animal species are usually used according to the different experimental purposes because of showing different immunological and pathological aspects. However, mice are commonly used because of their small size and the adequacy for studying immunological interaction and progress. Different mouse strains can be used because of their small size [13-16]. Rhotries produce two types of proteins; rhoptry proteins (ROPs) which have numerous targets in the infected cells [17]. The crucial role of T cells against *T. gondii* infection has been demonstrated in a number of studies. It was also shown that the cytotoxic CD8+ T cells produced IFN-γ and interleukin-2 (IL-2) [18,19]. Added to the cytotoxic T cells, the helper T cells are also effective against toxoplasmosis. They are generally grouped into T Helper 1 (Th1) and T Helper 2 (Th2) subpopulations based on the type of cytokines they produce. The Th1 cells secrete IFN-γ, interleukin-2 and beta Tumor Necrosis Factor (TNF-β) whereas the Th2 cells produce IL-4, IL-5, IL-10 and IL-13 [20]. Protective immunity against toxoplasmosis is predominantly attributed to a Th1 type of response [21]. However, antibodies also contribute to controlling the infection. For example, in *in vitro* study, specific antibodies against SAG1 could prevent the invasion of human fibroblast cells by tachyzoites [22]. In *in vivo*, antibodies might prevent the dissemination of extracellular stages via neutralization through opsonisation or complement activation [23,24].

Current status of vaccine development against toxoplasmosis

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

Recombinant DNA and protein as subunit vaccine

In the last few years, numerous vaccine studies have been focused on the use of recombinant subunit vaccines (DNA and protein subunit vaccine). Such kinds of vaccines have numerous advantages such as the induction of long-lasting immunity, high safety, and low costs. In the case of DNA vaccines, the target gene of *T. gondii* is inserted into a eukaryotic vector which possesses the capacity to express the antigen inside the immunized host. While vaccination based on recombinant protein is depending on employing of a prepared parasite antigen, which is expressed in a prokaryotic or eukaryotic vector in each host cell.
in a preceding stage. In the last decade, both recombinant DNA and protein vaccines have been achieved significant advances in triggering potent immune responses and inducing high levels of protection. Additionally, a tremendous advance in the manufacturing of recombinant protein vaccines has been occurred by using adjuvant substances to targeted vaccine antigens [27].

**Conclusion**

In conclusion, the data represented in this review are reporting promising results regarding the vaccination trials with recombinant subunit vaccines against *T. gondii*. This data can be exploited in the development of effective and safe vaccine and its implementation in large animals or clinical trials. Not only antigens derived from essential *T. gondii* organelles but also those contributed to metabolic or vital processes could be used.

### Acknowledgments

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### Tables

<table>
<thead>
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<th><em>Toxoplasma</em> antigen</th>
<th>Experimental animal, challenge strain and protection index</th>
<th>Year [Reference]</th>
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</thead>
<tbody>
<tr>
<td>Bradyzoite antigen (BAG), Matrix antigen (MAG)</td>
<td>- C3H/HeN mice&lt;br&gt;- Avirulent <em>T. gondii</em> SSI 119 strain&lt;br&gt;- Reduced cyst formation</td>
<td>2006 [28]</td>
</tr>
<tr>
<td>Apical membrane antigen 1 (AMA1)</td>
<td>- BALB/c &amp; C57BL/6 mice&lt;br&gt;- Avirulent <em>T. gondii</em> Beverley strain (type II)&lt;br&gt;- Survival rate (60%) &amp; (40%) respectively</td>
<td>2007 [29]</td>
</tr>
<tr>
<td>Dense granule 1 (GRA1)</td>
<td>- BALB/c mice&lt;br&gt;- RH (type I)&lt;br&gt;- Prolonged survival time</td>
<td>2007 [30]</td>
</tr>
<tr>
<td>Rhooptry 13 (ROP13)</td>
<td>- Kunming mice&lt;br&gt;- RH&lt;br&gt;- Prolonged survival time</td>
<td>2012 [31]</td>
</tr>
<tr>
<td>Immune mapped protein-1 (TgMP1)</td>
<td>- BALB/c mice&lt;br&gt;- RH&lt;br&gt;- Prolonged survival time</td>
<td>2012 [32]</td>
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<tr>
<td>Surface antigen 1 (SAG1) and 14–3–3</td>
<td>- Kunming mice&lt;br&gt;- RH&lt;br&gt;- Prolonged survival time</td>
<td>2012 [33]</td>
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<tr>
<td>AMA1</td>
<td>- C57BL/6 mice&lt;br&gt;- PLK (type II)&lt;br&gt;- 35%</td>
<td>2012 [34]</td>
</tr>
<tr>
<td>Cyclophilin (Cyp)</td>
<td>- BALB/c mice&lt;br&gt;- RH&lt;br&gt;- 37.5%</td>
<td>2013 [35]</td>
</tr>
<tr>
<td>Microneme 11 (MIC11)</td>
<td>- BALB/c mice&lt;br&gt;- RH&lt;br&gt;- 20%</td>
<td>2013 [36]</td>
</tr>
<tr>
<td>MIC3, ROP18</td>
<td>- ICR mice&lt;br&gt;- RH&lt;br&gt;- Prolonged survival time</td>
<td>2013 [37]</td>
</tr>
<tr>
<td>Calcium dependent protein kinase 3 (TgCDPK3)</td>
<td>- Kunming mice&lt;br&gt;- RH&lt;br&gt;- Prolonged survival</td>
<td>2013 [38]</td>
</tr>
<tr>
<td>ROP9</td>
<td>- Kunming mice&lt;br&gt;- RH&lt;br&gt;- Prolonged survival</td>
<td>2014 [39]</td>
</tr>
<tr>
<td>Deoxyribose Phosphate Aldolase (TgDPA)</td>
<td>- Swiss Webster (SW) mice&lt;br&gt;- RH&lt;br&gt;- Prolonged survival</td>
<td>2014 [40]</td>
</tr>
<tr>
<td>Protein/Proteins</td>
<td>Animal Species</td>
<td>Strain</td>
</tr>
<tr>
<td>---------------------------------------------------</td>
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<tr>
<td>Glutathione reductase protein</td>
<td>Swiss Webster mice</td>
<td>RH</td>
</tr>
<tr>
<td>Glutathione-S-transferase (TgGST)</td>
<td>Swiss Webster mice</td>
<td>RH</td>
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<tr>
<td>SAG1, GRA2, GRA7 and ROP16</td>
<td>BALB/c mice</td>
<td>RH</td>
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<tr>
<td>ROPS/ROP7</td>
<td>BALB/c mice</td>
<td>PRU (Type II) and RH strain</td>
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<tr>
<td>ROP1</td>
<td>BALB/c mice</td>
<td>RH</td>
</tr>
<tr>
<td>GRA1, MIC3</td>
<td>BALB/c mice</td>
<td>RH</td>
</tr>
<tr>
<td>Secreted protein with an altered thrombospondin repeat (TgSPATR)</td>
<td>BALB/c mice</td>
<td>RH</td>
</tr>
<tr>
<td>Superoxide dismutase (TgSOD)</td>
<td>BALB/c mice</td>
<td>ME49 (Type II) strain</td>
</tr>
<tr>
<td>ROP18, perforin-like protein 1 (PLP1)</td>
<td>Kunming mice</td>
<td>PRU</td>
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Table 2: Vaccine studies in which recombinant protein has been used as a vaccine candidate

<table>
<thead>
<tr>
<th>Toxoplasma antigen</th>
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<tr>
<td>SAG1 (Protein+ complete Freund's adjuvant)</td>
<td>- Dunkin Hartley guinea pigs - 76K (type II) and C56 (type III) strain - Reduced parasite burden in internal organs</td>
<td>2006 [59]</td>
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<tr>
<td>GRA1 (Protein + PROVAXTM adjuvant)</td>
<td>- BALB/c mice - RH - Prolonged survival</td>
<td>2007 [30]</td>
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<td>GRA2, GRA6 (Protein + monophosphoryl lipid A adjuvant)</td>
<td>- CBA/J mice - PRU - Reduced cyst formation</td>
<td>2007 [60]</td>
</tr>
<tr>
<td>ROP2, ROP4</td>
<td>- C3H/HeJ mice - Low virulent DX T. gondii strain (type II) - Reduced cyst formation</td>
<td>2009 [61]</td>
</tr>
<tr>
<td>Actin depolymerizing factor protein</td>
<td>- BALB/c mice - RH - Prolonged survival</td>
<td>2012 [62]</td>
</tr>
<tr>
<td>SAG1 (Protein + poly lactide-co-glycolide)</td>
<td>- BALB/c mice - RH - 20%</td>
<td>2013 [63]</td>
</tr>
<tr>
<td>ROP5</td>
<td>- BALB/c mice - RH - Prolonged survival</td>
<td>2013 [64]</td>
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<tr>
<td>ROP18 (Protein + ginsenoside Re as adjuvant)</td>
<td>- ICR mice - RH - Prolonged survival</td>
<td>2013 [65]</td>
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<tr>
<td>Protein Disulfide Isomerase (TgPDI)</td>
<td>- BALB/c mice - RH (type I) - 35%</td>
<td>2013 [66]</td>
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<tr>
<td>Profilin (PF) (Protein + Oligomannose-coated liposome adjuvant (OML))</td>
<td>- C57BL/6 mice - PLK (type II) - 66.7%</td>
<td>2014 [67]</td>
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<tr>
<td>ROP18, ROP38 (Protein + poly (lactide-co-glycolide(PLG)</td>
<td>- Kunming mice - PRU - Reduce brain cyst number</td>
<td>2015 [68]</td>
</tr>
<tr>
<td>ROP5, ROP18 (Protein +poly I:C adjuvant)</td>
<td>-BALB/c and C3H/HeOuJ mice -DX and RH strain - Reduce brain cyst number in DX and prolonged survival in RH</td>
<td>2015 [69]</td>
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<tr>
<td>MIC1, 4, 6</td>
<td>-C57BL/6 - ME49 and RH strains -Reduce brain cyst number in ME49 and prolonged survival in RH</td>
<td>2015 [70]</td>
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<td>Phosphoglycerate mutase 2 (TgPGAM 2)</td>
<td>-BALB/c mice - RH strain - Prolonged survival</td>
<td>2016 [71]</td>
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<tr>
<td>TgCPDK6, ROP18 (Protein + poly(lactide-co-glycolide) microspheres)</td>
<td>- Kunming mice - PRU and RH strains -Reduce brain cyst number in PRU and prolonged survival in RH</td>
<td>2016 [72]</td>
</tr>
<tr>
<td>Peroxiredoxin 3 (TgPrx3)</td>
<td>- C57BL/6 - PLK - 55.6%</td>
<td>2016 [73]</td>
</tr>
<tr>
<td>Actin depolymerizing factor (TgADF)</td>
<td>-BALB/c mice - RH - Prolonged survival</td>
<td>2016 [74]</td>
</tr>
</tbody>
</table>
SAG1, GRA2 (Protein +Poly (DL-lactide-co-glycolide) (PLGA) microspheres (MS))
- BALB/c mice
- RH
- Prolonged survival
2016 [75]

Aspartic protease 3 (ASP-3)
- BALB/c mice
- RH
- Prolonged survival
2017 [76]

Elongation factor 1-alpha rTgEF-1α (Protein + Freund adjuvant)
- BALB/c mice
- RH
- Prolonged survival
2017 [77]

TgPrx1
- C57BL/6
- PLK
- 66.7%
2017 [78]

Heat shock protein 70 (TgHSP70) (Protein +Alum)
- C57BL/6
- ME49
- Reduce brain inflammation
2017 [79]

TgPi-1+ROP2, TgPi-1+GRA4, TgPi-1+ROP2+GRA4
- C3H/HeN mice
- ME49
- Reduce brain cyst number
2018 [80]

ROP2 + ROP4 + SAG1 + MAG1 (Protein + Monophosphoryl lipid A from *Salmonella enterica* and Alhydrogel (InvivoGen))
- C3H/HeOuJ mice
- DX
- Reduce parasite cyst in brain and neurological severity
2018 [81]

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