Development of Nanovaccine against *Toxoplasma gondii*

Subjects: Infectious Diseases

Contributor: Carina Brito , Camila Lourenço , Joana Magalhães , Salette Reis , Margarida Borges

Nanoparticles include particles ranging in size from nanometers to micrometers, whose physicochemical characteristics are optimized to make them appropriate delivery vehicles for drugs or immunogens important in the fight and/or prevention of infectious diseases. There has been a rise in the use of nanoparticles in preventive vaccine formulations as immunostimulatory adjuvants, and as vehicles for immunogen delivery to target immune cells. *Toxoplasma* is important worldwide, and may cause human toxoplasmosis. In immunocompetent hosts, infection is usually asymptomatic, but in immunocompromised patients it can cause serious neurological and ocular consequences, such as encephalitis and retinochoroiditis. Primary infection during pregnancy may cause abortion or congenital toxoplasmosis. There is no effective human vaccine against this disease. Evidence has emerged from several experimental studies testing nanovaccines showing them to be promising tools in the prevention of experimental toxoplasmosis.

nanoparticles Toxoplasma gondii adjuvant immune system toxoplasmosis

1. Introduction

Toxoplasma gondii (*T. gondii*), has a worldwide distribution [1]. *T. gondii* is an intracellular protozoan parasite of the Apicomplexa phylum, which may cause significant clinical manifestations of toxoplasmosis, especially in immunocompromised individuals, pregnant women, and cattle [2]. It has a complex life cycle (**Figure 1**) involving sexual reproduction in cats, the definitive host, and asexual reproduction in other warm-blooded animals, the intermediate hosts. There are three infectious development stages: tachyzoites, bradyzoites (in tissue cysts), and sporozoites (within oocysts) [3]. The parasite can be transmitted horizontally or vertically [4]. Parasite reactivation or primary infection during pregnancy can cause congenital toxoplasmosis, leading to severe consequences for the fetus, such as abortion, mental retardation, ocular disease, and hydrocephaly [5]. Immunocompromised patients may also develop severe diseases, such as encephalitis and pneumonitis. Some psychiatric illnesses such as schizophrenia, depression, and bipolar disorder have been associated with *T. gondii* infection [6][7].

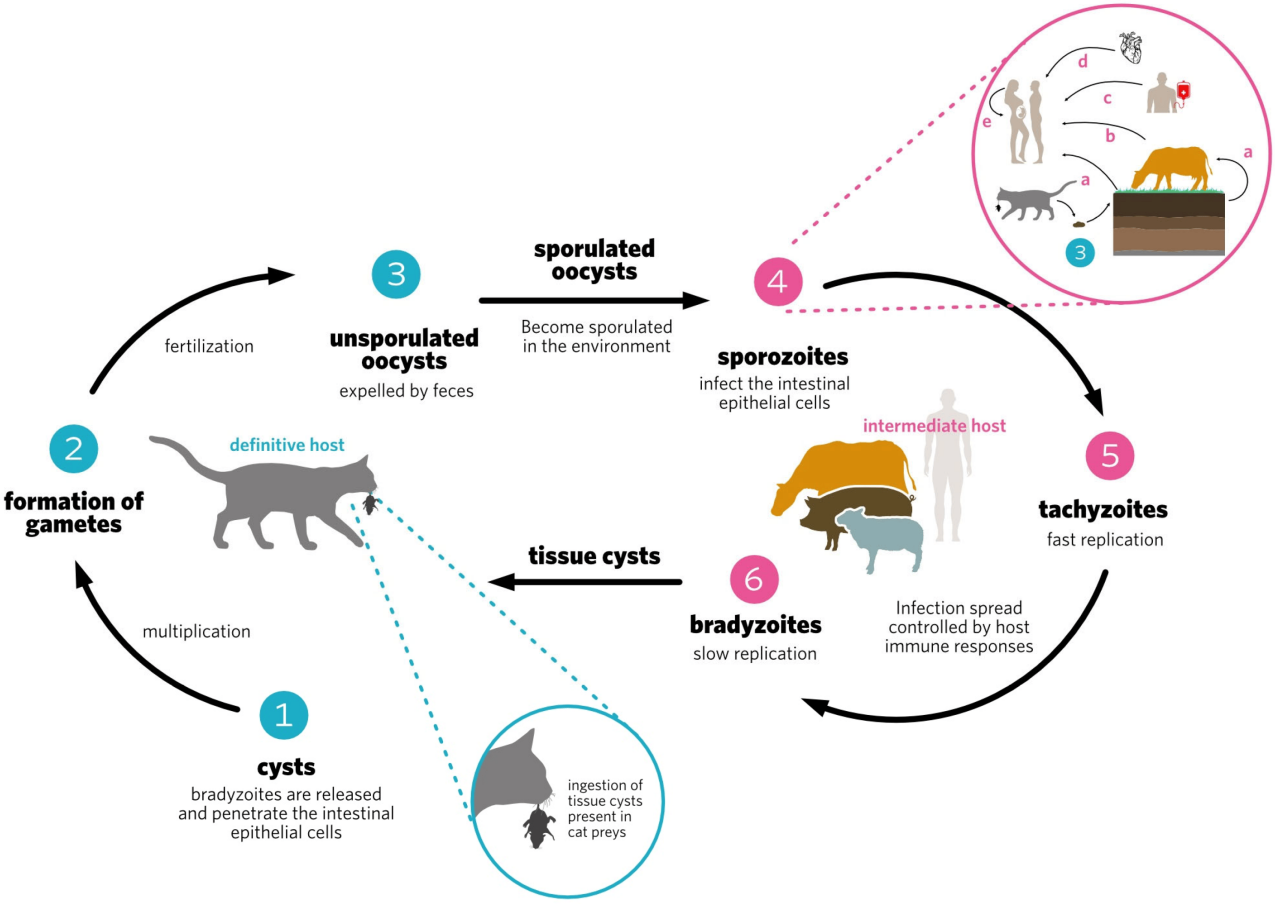

Figure 1. Life cycle of *T. gondii*. 1—Consumption of meat containing cysts by the definitive host (Felidae), followed by the release of bradyzoites, and infection of intestinal epithelial cells; 2—After intense multiplication, gametes are formed; 3—After fertilization, unsporulated oocysts are released in the feces of the definitive host; 4—Intermediate hosts are infected by diverse means, such as by the ingestion of sporulated oocysts (**a**) and consequently the sporozoites infect the intestinal cells; 5—Conversion of sporozoites into tachyzoites, the rapidly multiplying form; and 6—Host immune responses contribute to the conversion of tachyzoites into bradyzoites, constituting cysts, a slowly replicating form. There are several pathways of intermediate host transmission: (**a**) ingestion of oocysts present in water, vegetables, or fruits; (**b**) ingestion of tissue cysts present in undercooked meat; (**c**) infection with tachyzoites by blood transfusion; (**d**) infection with cysts through tissue transplantation; and by (**e**) vertical transmission.

Different drugs are used in conventional toxoplasmosis treatment, such as pyrimethamine and sulfadiazine. However, therapeutic adherence of this drug combination is still low since it exhibits severe side-effects and is only active against the tachyzoite form, failing to eliminate the latent forms, such as slow-dividing bradyzoites within tissue cysts [8]. Vaccines are a good alternative to chemical therapeutics. However, it has been difficult to achieve an effective, durable, and safe vaccine against toxoplasmosis. Nowadays, only one vaccine, Toxovax® [9], is licensed for use in sheep and goats. This live attenuated vaccine has some disadvantages including limited shelf life, risk of infection to humans handling the vaccine, and possible virulence reversion [10].

New approaches are needed for human toxoplasmosis prevention. With the rapid development of nanotechnology in biomedicine, nanoparticles (NPs) have become attractive and strong candidates for the prevention of infectious diseases, such as COVID-19, hepatitis B, and toxoplasmosis, among many others [11][12]. In some cases, NPs are immunogenic by themselves, without the need for adjuvants to activate the immune system [13][14].

2. Benefits of Using Nanoparticles in Vaccination

NPs are used as an antigen delivery tool and/or as an immune-stimulant adjuvant to enhance immunity against several pathogens such as *Mycobacterium tuberculosis* and *T. gondii* [15][16].

In traditional vaccines, the formulation is distributed without a specific target in the body. NPs as a vehicle may alter the active substance distribution *in vivo*, since they can be covered with antibodies on their surface, which are capable of recognizing cell-specific receptors, thus, allowing targeted delivery to a desired cell population and preventing potential damage to other cells, and consequently to other tissues [17]. Additionally, these NPs have a depot effect, when administered by intramuscular route, keeping the antigen in the tissue area adjacent to the administration site long enough to exert the necessary function. This allows a gradual release of the antigen, thereby increasing the exposure time to the immunogen by antigen-presenting cells (APCs). Therefore, APCs will increase their ability to present the antigen and induce an efficient T-cell response [18][19].

Adjuvants are chemical or biological compounds that stimulate the immune system against the administered antigen, thus, increasing the effectiveness of the vaccine [20]. Among the most common adjuvants, aluminum (Alum)-based compounds, such as amorphous Alum hydroxy-phosphate sulphate (AAHS), Alum hydroxide, Alum phosphate, and potassium Alum sulphate, are the most used in conventional human vaccines [21]. Synthetic oligodeoxynucleotides (ODNs) are also adjuvants; they contain unmethylated CpG motifs and can trigger cells expressing TLR9 inducing a Th1 response and proinflammatory cytokines. Overall, ODNs improve APC function and boost the humoral and cellular vaccine-specific immune responses [22]. However, these adjuvants have several disadvantages, such as the need to be stored at low temperatures or the possibility of allergic reactions at the injection site [23]. NPs are an alternative to the use of such adjuvants, with equal or high immune system-stimulating ability [20][24].

Since NPs share structural and size characteristics with viruses and bacteria they can mimic the process of a natural infection increasing the uptake of antigens by APCs, and consequently immune response initiation [17]. Studies have shown that macrophages and DCs are capable of capturing cationic NPs since their positive charge is attracted by the negative charge of the membrane surface of these cells [25]. The NPs can also be conjugated with antibodies specific to cell receptors, as previously mentioned, enabling NP internalization, as was shown for Herceptin-coated gold NPs endocytosed after interaction with the membrane HER2 receptor, used in breast cancer [26].

NPs are also used to improve the solubility of hydrophobic compounds, thus, obtaining a solution for parenteral administration, preventing antigen degradation and allowing the stabilization of a wide range of therapeutic agents

such as proteins, peptides, and nucleic acids, which leads to a reduction in doses of effective vaccines [20].

3. Recent Advances in the Use of Nanoparticles for *Toxoplasma gondii* Vaccination

A search using the terms “Nanoparticles”, “Vaccine”, and “*Toxoplasma gondii*” and the filter “last 10 years” enabled 40 articles to be identified. Among these, 16 corresponded to reviews concerning experimental NPs tested for the diagnosis and treatment of toxoplasmosis. Researchers found 24 articles detailing studies focused on the development of nanovaccines against *T. gondii* infection.

Several studies have been carried out into the development of a vaccine against *T. gondii*. However, an effective vaccine able to confer effective immunity against latent infection (elimination of tissue cysts) remains a challenge [27]. Recently, new approaches have been made in vaccination strategies, such as the use of NPs, which have been assessed mostly in rodents, showing promising results [28]. An ideal vaccine to control toxoplasmosis and prevent the development of chronic tissue cysts should induce a Th1- type immune response, since it has been shown that the INF-γ-secreting $CD8^+$ T lymphocyte is the main immune cell population involved in the long-term protective immunity against this disease [27]. Mucosal immunization routes, such as intranasal and/or intraoral, have been shown to induce effective protection when compared to systemic immunization routes, such as intramuscular or intravenous [29].

NP development has emerged as a novel vaccine platform, currently constituting a strategy to protect antigens from proteolytic degradation, ensuring a successful uptake by cells and inducing an effective immune response [28]. Different NP antigen delivery strategies have been studied, such as DNA and ribonucleic acid (RNA) vaccines, as well as protein and recombinant subunit vaccines [28]. Nowadays, DNA and RNA vaccines have been shown to be the most efficient platforms, able to induce *anti-T. gondii* immune responses, and easily produced at a low cost [30]. Indeed, calcium phosphate NPs (CaPNs) encapsulated with DNA or RNA coding for dense granule protein 14 (GRA14) have been shown to increase *T. gondii* specific IgG1 and IgG2a antibody responses and lymphocyte proliferation [31]. Similar NPs coding dense granule protein 7 (GRA7) also showed a strong cellular immune response, with a higher IgG2a-to-IgG1 ratio and higher IL-12 and INF-γ production [32]. Immunization using the mice model, with a modified dendrimer vaccine with mRNA replicons encoding dense granule protein 6 (GRA6), rhoptry protein 2A (ROP2A), rhoptry protein 18 (ROP18), surface antigen 1 (SAG1), surface antigen 2 (SAG2) and apical membrane antigen 1 (AMA1), led to protection against lethal infection [33]. It has been shown that a significant percentage of mice immunized with lipid nanoparticles encapsulated with nucleoside-triphosphatase II (NTPase II) survived post-challenge with *T. gondii* parasites [34]. Other similar lipid nanoparticles encapsulated with a plasmid encoding GRA15 led to a significantly higher production of specific IgG1 and IG2c antibodies and consequently higher survival rate compared to the controls [35]. Cocktail DNA vaccines of pcROM4 + pcGRA14 coated with CaPNs boosted immune responses and increased the protective efficacy against acute toxoplasmosis compared to cocktail DNA vaccine without CaPNs [36].

Protein and recombinant subunit vaccines are also extremely safe with low side effects since proteins are highly purified. A wide array of antigens has been tested, ranging from antigenic epitopes to total *T. gondii* antigenic extract (TE) [28]. Mice immunized with porous NPs containing TE have been shown to induce a Th1/Th17 immune response able to prolong mouse survival and drastically reduce brain cyst counts [37]. Polymeric NPs loaded with *T. gondii* histone H2A1 conferred mice with protection against infection, prolonging their survival and the production of Th1 cytokines [38]. A similar study using PLGA NPs containing T and B cell epitopes of AMA1, GRA4, ROP2, and SAG1, adjuvanted with potassium Alum sulphate, induced a stronger Th1 immune response in mice, compared to immunization solely with antigens [39]. Another study showed that intranasal immunization of mice with maltodextrin-based NPs (DGNPs) containing TE conferred significant protection against chronic and congenital toxoplasmosis [40]. This vaccine was later shown to induce protection against latent toxoplasmosis and transplacental transmission using the sheep model [41]. Some immunization studies with different types of nanoparticles (CaPNs, PLGA, and Chitosan) encapsulated with a variety of *T. gondii* recombinant proteins, namely MIC3, ROP8, and SAG1, showed similar results, with higher levels of specific IgA and IgG2a, leading to a Th1 response and consequently increased survival rate [42][43][44][45][46]. In addition to the use of parasite membrane proteins, the excretory secretory antigens (ESA) of *T.gondii* have also been used for vaccine development, since they have proved to play important roles in the immune escape and pathogenesis of the parasite, and the results showed increased levels of IFN-γ and IgG, as well as a reduction in the parasite load [47][48][49].

Studies concerning *T. gondii* describing the use of virus-like particles (VLPs) are limited. Nevertheless, some VLP-based vaccines are already commercially available for several human viral diseases, such as Epaxal® for hepatitis A virus, Gardasil® for human papillomavirus, and GenHevac B® for hepatitis B virus, among others [50]. The advantages of VLPs described include: safety; small size, allowing rapid traffic into the lymph nodes and consequent induction of a prompt immune response; and their repetitive antigen presentation, promoting a powerful immune response [51]. It has been shown that immunization with VLPs containing *T. gondii* inner membrane complex subcompartment protein 3 (IMC ISP3) with influenza matrix protein 1 (M1) as a core protein conferred mice with protection against *T. gondii* ME49 infection [52]. Thereafter, multiple studies have shown the efficacy of VLP vaccines containing different antigens in enabling protection against the ME49 strain. Finally, VLPs expressing ROP4 or ROP13 conferred complete survival against the challenge with the ME49 strain and reduced cyst numbers [53]. It must be highlighted that these VLPs were developed containing the self-assembling viral protein M1 determinant for VLP generation in which M1 is the main force for viral budding and particle formation. It is assumed that *T. gondii* proteins will be on the surface of VLPs [52][53].

It is important to mention that almost all studies of nanovaccines in toxoplasmosis used the mouse as a study model. Therefore, future pre-clinical trials should be extended to other animal models. There should also be a harmonization of the immune parameters assessed in these studies in order to support the studies carried out.

References

1. Dubey, J.P. Outbreaks of clinical toxoplasmosis in humans: Five decades of personal experience, perspectives and lessons learned. Parasites Vectors 2021, 14, 263.
2. Deng, H.; Devleesschauwer, B.; Liu, M.; Li, J.; Wu, Y.; van der Giessen, J.W.B.; Opsteegh, M. Seroprevalence of Toxoplasma gondii in pregnant women and livestock in the mainland of China: A systematic review and hierarchical meta-analysis. Sci. Rep. 2018, 8, 6218.
3. Delgado, I.L.S.; Zúquete, S.; Santos, D.; Basto, A.P.; Leitão, A.; Nolasco, S. The Apicomplexan Parasite Toxoplasma gondii. Encyclopedia 2022, 2, 189–211.
4. Milne, G.; Webster, J.P.; Walker, M. Toward Improving Interventions Against Toxoplasmosis by Identifying Routes of Transmission Using Sporozoite-specific Serological Tools. Clin. Infect. Dis. 2020, 71, e686–e693.
5. Elsheikha, H. Congenital toxoplasmosis: Priorities for further health promotion action. Public Health 2008, 122, 335–353.
6. Wang, Z.-D.; Wang, S.-C.; Liu, H.-H.; Ma, H.-Y.; Li, Z.-Y.; Wei, F.; Zhu, X.-Q.; Liu, Q. Prevalence and burden of Toxoplasma gondii infection in HIV-infected people: A systematic review and meta-analysis. Lancet HIV 2017, 4, e177–e188.
7. Liu, T.; Gao, P.; Bu, D.; Liu, D. Association between Toxoplasma gondii infection and psychiatric disorders: A cross-sectional study in China. Sci. Rep. 2022, 12, 15092.
8. Silva, M.d.; Teixeira, C.; Gomes, P.; Borges, M. Promising Drug Targets and Compounds with Anti-Toxoplasma gondii Activity. Microorganisms 2021, 9, 1960.
9. Brito, C.; Silva, T.M.; Castro, M.M.; Wyrwas, W.; Oliveira, B.; Fonseca, B.M.; Oliveira, P.; Roberts, C.W.; Teixeira, N.; Borges, M. Toxoplasma gondii infection reduces serum progesterone levels and adverse effects at the maternal-foetal interface. Parasite Immunol. 2020, 42, e12690.
10. Hasan, T.; Nishikawa, Y. Advances in vaccine development and the immune response against toxoplasmosis in sheep and goats. Front. Veter-Sci. 2022, 9, 951584.
11. Peplow, M. Nanotechnology offers alternative ways to fight COVID-19 pandemic with antivirals. Nat. Biotechnol. 2021, 39, 1172–1174.
12. Rasmussen, M.K.; Kardjilov, N.; Oliveira, C.L.P.; Watts, B.; Villanova, J.; Botosso, V.F.; Sant'Anna, O.A.; Fantini, M.C.A.; Bordallo, H.N. 3D visualisation of hepatitis B vaccine in the oral delivery vehicle SBA-15. Sci. Rep. 2019, 9, 6106.
13. Adeyemi, O.S.; Murata, Y.; Sugi, T.; Han, Y.; Kato, K. Nanoparticles show potential to retard bradyzoites in vitro formation of Toxoplasma gondii. Folia Parasitol. 2019, 66, 001.
14. Dacoba, T.G.; Olivera, A.; Torres, D.; Crecente-Campo, J.; Alonso, M.J. Modulating the immune system through nanotechnology. Semin. Immunol. 2017, 34, 78–102.

15. Pati, R.; Shevtsov, M.; Sonawane, A. Nanoparticle Vaccines Against Infectious Diseases. Front. Immunol. 2018, 9, 2224.
16. Petkar, K.C.; Patil, S.M.; Chavhan, S.S.; Kaneko, K.; Sawant, K.K.; Kunda, N.K.; Saleem, I.Y. An Overview of Nanocarrier-Based Adjuvants for Vaccine Delivery. Pharmaceutics 2021, 13, 455.
17. Luo, M.; Samandi, L.; Wang, Z.; Chen, Z.; Gao, J. Synthetic nanovaccines for immunotherapy. J. Control. Release 2017, 263, 200–210.
18. Smith, D.M.; Simon, J.K.; Baker, J.R., Jr. Applications of nanotechnology for immunology. Nat. Rev. Immunol. 2013, 13, 592–605.
19. Henriksen-Lacey, M.; Christensen, D.; Bramwell, V.W.; Lindenstrøm, T.; Agger, E.M.; Andersen, P.; Perrie, Y. Liposomal cationic charge and antigen adsorption are important properties for the efficient deposition of antigen at the injection site and ability of the vaccine to induce a CMI response. J. Control. Release 2010, 145, 102–108.
20. Hayat, S.M.G.; Darroudi, M. Nanovaccine: A novel approach in immunization. J. Cell. Physiol. 2019, 234, 12530–12536.
21. Djurisic, S.; Jakobsen, J.C.; Petersen, S.B.; Kenfelt, M.; Gluud, C. Aluminium adjuvants used in vaccines versus placebo or no intervention. Cochrane Database Syst. Rev. 2017, 2017, CD012805.
22. Bode, C.; Zhao, G.; Steinhagen, F.; Kinjo, T.; Klinman, D.M. CpG DNA as a vaccine adjuvant. Expert Rev. Vaccines 2011, 10, 499–511.
23. Mbow, M.L.; De Gregorio, E.; Valiante, N.M.; Rappuoli, R. New adjuvants for human vaccines. Curr. Opin. Immunol. 2010, 22, 411–416.
24. Swaminathan, G.; Thoryk, E.A.; Cox, K.S.; Meschino, S.; Dubey, S.A.; Vora, K.A.; Celano, R.; Gindy, M.; Casimiro, D.R.; Bett, A.J. A novel lipid nanoparticle adjuvant significantly enhances B cell and T cell responses to sub-unit vaccine antigens. Vaccine 2016, 34, 110–119.
25. Lin, J.; Alexander-Katz, A. Cell Membranes Open “Doors” for Cationic Nanoparticles/Biomolecules: Insights into Uptake Kinetics. ACS Nano 2013, 7, 10799–10808.
26. Juan, A.; Cimas, F.J.; Bravo, I.; Pandiella, A.; Ocaña, A.; Alonso-Moreno, C. An Overview of Antibody Conjugated Polymeric Nanoparticles for Breast Cancer Therapy. Pharmaceutics 2020, 12, 802.
27. Lim, S.S.-Y.; Othman, R.Y. Recent Advances in Toxoplasma gondii Immunotherapeutics. Korean J. Parasitol. 2014, 52, 581–593.
28. Chu, K.-B.; Quan, F.-S. Advances in Toxoplasma gondii Vaccines: Current Strategies and Challenges for Vaccine Development. Vaccines 2021, 9, 413.

29. Wang, T.; Yin, H.; Li, Y.; Zhao, L.; Sun, X.; Cong, H. Vaccination with recombinant adenovirus expressing multi-stage antigens of Toxoplasma gondii by the mucosal route induces higher systemic cellular and local mucosal immune responses than with other vaccination routes. Parasite 2017, 24, 12.
30. Li, Y.; Zhou, H. Moving towards improved vaccines for Toxoplasma gondii. Expert Opin. Biol. Ther. 2018, 18, 273–280.
31. Ahmadpour, E.; Sarvi, S.; Soteh, M.B.H.; Sharif, M.; Rahimi, M.T.; Valadan, R.; Tehrani, M.; Khalilian, A.; Montazeri, M.; Ramandi, M.F.; et al. Enhancing immune responses to a DNA vaccine encoding Toxoplasma gondii GRA14 by calcium phosphate nanoparticles as an adjuvant. Immunol. Lett. 2017, 185, 40–47.
32. Sun, H.-C.; Huang, J.; Fu, Y.; Hao, L.-L.; Liu, X.; Shi, T.-Y. 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.
33. Chahal, J.S.; Khan, O.F.; Cooper, C.L.; McPartlan, J.S.; Tsosie, J.K.; Tilley, L.D.; Sidik, S.M.; Lourido, S.; Langer, R.; Bavari, S.; et al. Dendrimer-RNA nanoparticles generate protective immunity against lethal Ebola, H1N1 influenza, and Toxoplasma gondii challenges with a single dose. Proc. Natl. Acad. Sci. USA 2016, 113, E4133–E4142.
34. Luo, F.; Zheng, L.; Hu, Y.; Liu, S.; Wang, Y.; Xiong, Z.; Hu, X.; Tan, F. Induction of Protective Immunity against Toxoplasma gondii in Mice by Nucleoside Triphosphate Hydrolase-II (NTPase-II) Self-amplifying RNA Vaccine Encapsulated in Lipid Nanoparticle (LNP). Front Microbiol 2017, 8, 605.
35. Hasan, T.; Kawanishi, R.; Akita, H.; Nishikawa, Y. Toxoplasma gondii GRA15 DNA Vaccine with a Liposomal Nanocarrier Composed of an SS-Cleavable and pH-Activated Lipid-like Material Induces Protective Immunity against Toxoplasmosis in Mice. Vaccines 2021, 10, 21.
36. Rahimi, M.T.; Sarvi, S.; Sharif, M.; Abediankenari, S.; Ahmadpour, E.; Valadan, R.; Ramandie, M.F.; Hosseini, S.-A.; Daryani, A. Immunological evaluation of a DNA cocktail vaccine with co-delivery of calcium phosphate nanoparticles (CaPNs) against the Toxoplasma gondii RH strain in BALB/c mice. Parasitol. Res. 2016, 116, 609–616.
37. Dimier-Poisson, I.; Carpentier, R.; N'Guyen, T.T.L.; Dahmani, F.; Ducournau, C.; Betbeder, D. Porous nanoparticles as delivery system of complex antigens for an effective vaccine against acute and chronic Toxoplasma gondii infection. Biomaterials 2015, 50, 164–175.
38. Yu, Z.; Zhou, T.; Luo, Y.; Dong, L.; Li, C.; Liu, J.; Luo, J.; Yan, R.; Xu, L.; Song, X.; et al. Modulation Effects of Toxoplasma gondii Histone H2A1 on Murine Macrophages and Encapsulation with Polymer as a Vaccine Candidate. Vaccines 2020, 8, 731.

39. Roozbehani, M.; Falak, R.; Mohammadi, M.; Hemphill, A.; Razmjou, E.; Meamar, A.R.; Masoori, L.; Khoshmirsafa, M.; Moradi, M.; Gharavi, M.J. Characterization of a multi-epitope peptide with selective MHC-binding capabilities encapsulated in PLGA nanoparticles as a novel vaccine candidate against Toxoplasma gondii infection. Vaccine 2018, 36, 6124–6132.
40. Ducournau, C.; Nguyen, T.T.; Carpentier, R.; Lantier, I.; Germon, S.; Précausta, F.; Pisella, P.-J.; Leroux, H.; Van Langendonck, N.; Betbeder, D.; et al. Synthetic parasites: A successful mucosal nanoparticle vaccine against Toxoplasma congenital infection in mice. Futur. Microbiol. 2017, 12, 393–405.
41. Ducournau, C.; Moiré, N.; Carpentier, R.; Cantin, P.; Herkt, C.; Lantier, I.; Betbeder, D.; Dimier-Poisson, I. Effective Nanoparticle-Based Nasal Vaccine Against Latent and Congenital Toxoplasmosis in Sheep. Front. Immunol. 2020, 11, 2183.
42. Dodangeh, S.; Fasihi-Ramandi, M.; Daryani, A.; Valadan, R.; Asgarian-Omran, H.; Hosseininejad, Z.; Chegeni, T.N.; Pagheh, A.S.; Javidnia, J.; Sarvi, S. Protective efficacy by a novel multi-epitope vaccine, including MIC3, ROP8, and SAG1, against acute Toxoplasma gondii infection in BALB/c mice. Microb. Pathog. 2021, 153, 104764.
43. Nabi, H.; Rashid, I.; Ahmad, N.; Durrani, A.; Akbar, H.; Islam, S.; Bajwa, A.A.; Shehzad, W.; Ashraf, K.; Imran, N. Induction of specific humoral immune response in mice immunized with ROP18 nanospheres from Toxoplasma gondii. Parasitol. Res. 2016, 116, 359–370.
44. Naeem, H.; Sana, M.; Islam, S.; Khan, M.; Riaz, F.; Zafar, Z.; Akbar, H.; Shehzad, W.; Rashid, I. Induction of Th1 type-oriented humoral response through intranasal immunization of mice with SAG1-Toxoplasma gondii polymeric nanospheres. Artif. Cells Nanomed. Biotechnol. 2018, 46 (Suppl. S2), 1025–1034.
45. Allahyari, M.; Golkar, M.; Fard-Esfahani, P.; Dimier-Poisson, I.; Mévélec, M.-N. Co-delivery of PLGA nanoparticles loaded with rSAG1 antigen and TLR ligands: An efficient vaccine against chronic toxoplasmosis. Microb. Pathog. 2022, 162, 105312.
46. Khorshidvand, Z.; Khosravi, A.; Mahboobian, M.M.; Larki-Harchegani, A.; Fallah, M.; Maghsood, A.H. Novel naltrexone hydrochloride nanovaccine based on chitosan nanoparticles promotes induction of Th1 and Th17 immune responses resulting in protection against Toxoplasma gondii tachyzoites in a mouse model. Int. J. Biol. Macromol. 2022, 208, 962–972.
47. Gaafar, M.R.; El-Mansoury, S.T.; Eissa, M.M.; Shalaby, T.I.; Younis, L.K.; Rashed, H.A. Effect of alginate nanoparticles on the immunogenicity of excretory-secretory antigens against acute toxoplasmosis in murine model. Acta Trop. 2022, 225, 106215.
48. Abdollahi, S.H.; Shahmabadi, H.E.; Arababadi, M.K.; Askari, N.; Falahati-Pour, S.K. The immune response against Toxoplasma gondii in BALB/c mice induced by mannose-modified nanoliposome of excreted/secreted antigens. Parasitol. Res. 2021, 120, 2855–2861.

49. Azadi, Y.; Ahmadpour, E.; Hamishehkar, H.; Daryani, A.; Spotin, A.; Mahami-Oskouei, M.; Barac, A.; Rajabi, S.; Alizadeh, P.; Montazeri, M. Quantification of Toxoplasma gondii in the tissues of BALB/c mice after immunization with nanoliposomal excretory-secretory antigens using Real-Time PCR. Comp. Immunol. Microbiol. Infect. Dis. 2018, 59, 52–56.
50. Kushnir, N.; Streatfield, S.J.; Yusibov, V. Virus-like particles as a highly efficient vaccine platform: Diversity of targets and production systems and advances in clinical development. Vaccine 2012, 31, 58–83.
51. Chu, K.-B.; Quan, F.-S. Virus-Like Particle Vaccines Against Respiratory Viruses and Protozoan Parasites. Curr. Top. Microbiol. Immunol. 2021, 433, 77–106.
52. Lee, D.H.; Lee, S.H.; Kim, A.R.; Quan, F.S. Virus-Like Nanoparticle Vaccine Confers Protection against Toxoplasma gondii. PLoS ONE 2016, 11, e0161231.
53. Kang, H.-J.; Lee, S.H.; Kim, M.J.; Chu, K.B.; Lee, D.H.; Chopra, M.; Choi, H.J.; Park, H.; Jin, H.; Quan, F.-S. Influenza Virus-Like Particles Presenting both Toxoplasma gondii ROP4 and ROP13 Enhance Protection against T. gondii Infection. Pharmaceutics 2019, 11, 342.