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Supplementary Table 3. Baseline characteristics of included studies based on immunization experiments with protein vaccines against *T. gondii* in mouse models (single antigens)

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
ROP1	FCA and FIA	Protein (10 μg)+FCA for prime injection+FIA for boosters, s.c	BALB/c	1×10^3 Tachyzoites, RH strain, i.p	Induced humoral immune response ↑ SI (3.04±0.21, p<0.001) ↑ IFN-γ (1,457±31.19, p<0.001) and IL-4 (186±14.17, p<0.01)	NR	Increased survival rate (mean survival of 29 days, p<0.05)	These findings proposed that the ROP1 Ag is a potential candidate for the development of vaccine against toxoplasmosis. Complete protection may be achieved by combining ROP1 with other immunogenic rhoptry antigens.	[5]
ROP2	Alum	Protein (10 μg), i.m	C57BL/6 (H-2^b) and C3H (H-2^k).	20 (sublethal dose) or 100 (lethal dose) ME49 tissue cysts, orally	-	20 (sublethal dose) ME49 tissue cysts, orally C57BL/6: reduced (none significant) C3H: reduced (p<0.01)	100 (lethal dose) ME49 tissue cysts, orally There were no significant differences in the survival rates from both strains of immunized mice compared to the control groups	The results reinforce the value of alum as a possible adjuvant to be used in immunization against *T. gondii*, allowing the development of a vaccine for wide application for either humans or animals. We consider that combinations with other effective antigens that generate immunity by different strategies should also be taken into account in the future.	[32]
	CpG-ODN	Protein (10 μg)+CpG (10 μg), i.m	C3H/HeN (H-2^k)	20 (sublethal dose) tissue cysts, Me49 (Type II) strain, orally	Induced a strong humoral Th1-biased response High IgG2a to IgG1 antibody ratio ↑ IFN-γ and IL-10	Reduced (63%, p<0.001)	NR	Our results indicate that CpG-ODN is an important candidate adjuvant for use in potential vaccines against this pathogen.	[33]
	Quil-A	Protein (10 μg)+10 μg Quil-A, i.n	BALB/c	NR	↑ IgG (in 5/10 mice, 50%) and IgA (in 2/10 mice, 20%) antibodies in sera of mice at day 62 of the experiment Elicited significant lymphocyte proliferation response	NR	NR	These results indicate that intranasal immunization with recombinant protein ROP2 plus Quil-A can elicit both cellular and humoral immune responses in BALB/c mice.	[34]
ROP5	FCA and FIA	Protein (100 μg)+ FCA+2 boosters in FIA, s.c	BALB/c	1×10^2 Tachyzoites, RH strain, i.p	↑ Level of IgG antibodies (p<0.01) Induced mixed Th1/Th2 immune responses with the predominance of IgG2a over IgG1 ↑ IFN-γ, IL-2, IL-4, and IL-10 (p<0.05) ↑ Splenocyte proliferation (p<0.05)	NR	Prolonged survival time (p<0.05)	This study demonstrated the novel finding that ROP5 induced a strong protective humoral and cellular response against *T. gondii* infection, which indicated that it is a potential vaccine candidate against toxoplasmosis.	[35]

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Supplementary Table 3. Continued

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
ROP17	-	Proteins (15, 25, 35 or 45 μg of rTgROP17), i.n	BALB/c	1×10^4 and 4×10^4 tachyzoites of RH strain for chronic and acute assay, respectively, orally	↑ IgG antibody production of the mice immunized with 25, 35, and 45 μg rTgROP17 ($p<0.05$) ↑ IgG1 and IgG2a in the sera of all the mice immunized with rTgROP17, particularly in those immunized with 35 and 45 μg rTgROP17 ($p<0.05$) Mixed Th1/Th2 immune response (predominance of IgG2a over IgG1) ↑ Splenocyte proliferation ($p<0.01$) ↑ IFN-γ ($p<0.01$), IL-2 (p 0.01), IL-4 ($p<0.05$) ↑ Mucosal immune responses (SIgA antibody titers) in the nasal, vaginal and intestinal washes of rTgROP17-immunized mice ($p<0.01$)	↓ Liver and brain parasite burdens	Increased survival rate 30 days post challenge (75% protection, $p<0.01$)	The study suggests that intranasal immunization of mice with rTgROP17 can induce both systemic and local immune responses to provide protection against lethal *T. gondii* infection through reduction of the tachyzoite burdens in the host tissues and increases of the animal survivals. We conclude that ROP17 is a promising vaccine candidate against infection with *T. gondii*.	[36]
ROP18 Groups: ROP18S2+ Montanide™ ISA 71+poly I:C (s.c-M-P-ROP18), s.c ROP18S2+CT (i.n-CT-ROP18), i.n Control groups	Montanide ISA 71, poly (I:C) and CT	s.c and i.n	CBA/J ($H\text{-}2^k$)	60 Cysts of the 76 K strain, orally	Significantly higher specific IgG antibody in s.c-M-P-ROP18 group, compared with i.n-CT-ROP18 group ↑ Specific IgG antibody, compared to controls (especially in the s.c-M-P-ROP18 group) The predominance of IgG1 over the IgG2a ↑ Intestinal IgA response ↑ IFN-γ, IL-2, and IL-5 responses in immunized mice compared to the control groups ($p<0.05$) Mixed Th1/Th2 immune response with the predominance of Th1 Similar percentage of $CD8^+$ T cells between vaccinated and control groups ($p>0.05$)	Reduced for i.n-CT-ROP18 group (50%, $p<0.001$)	NR	These results suggest that ROP18 could be a component of a subunit vaccine against toxoplasmosis and that strategies designed to enhance mucosal protective immune responses could lead to more encouraging results.	[18]
ROP18	Re	Proteins (100 μg rROP18)+different dosages of Re (10, 50 or 100 μg), s.c	ICR	5×10^2 Tachyzoites, RH strain, i.p	↑ IgG antibody than control ($p<0.05$). Co-administration of rROP18 with Re (50 μg and 100 μg) induced numerically higher specific antibody level than that with10 μg Re ($p<0.05$) Mixed IgG1/IgG2a response, with the predominance of IgG1 production ↑ Splenocyte proliferation in mice immunized with rROP18+Re (50 μg and 100 μg, $p<0.05$) ↑ IFN-γ and IL-4 ($p<0.05$)	NR	Increased survival time (15 days in mice immunized with rROP18+Re, compared with 6 days in control, $p<0.05$)	The data demonstrate that by the addition of ginsenoside Re, the rROP18 triggered a stronger humoral and cellular response against *T. gondii*, and that Re is a promising vaccine adjuvant against toxoplasmosis, deserves further evaluation and development.	[37]

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https://doi.org/10.7774/cevr.2019.8.1.4 http://www.ecevr.org/

Supplementary Table 3. Continued

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
ROP18 Groups: G1. Montanide adjuvant only, i.p G2. 10 μg rROP18, i.p G3. 10 μg rROP18+ Montanide, i.p G4. PLGA only, i.n G5. rROP18-PLGA, i.n	Montanide and PLGA	Proteins (10 μg)+ PLGA, i.p and i.n	Swiss-Webster mice	NR	Significantly higher levels of IgG in mice immunized with rROP18-adjuvant, compared with rROP18 group ($p<0.05$) Significantly higher levels of IgG in mice immunized with rROP18-PLGA, compared with PLGA group ($p<0.05$) IgA levels in rROP18-PLGA were significant ($p<0.05$) as compared to PLGA The predominance of IgG2a over IgG1 in mice vaccinated with rROP18-PLGA ($p<0.05$) The predominance of IgG1 over IgG2a in mice vaccinated with rROP18-adjuvant ($p<0.05$)	NR	NR	It was concluded that nanospheres of ROP18 would be a non-invasive approach to develop vaccines against *T. gondii*. Further experiments are needed to determine the cellular response to these nanospheres in a mouse model for chronic toxoplasmosis.	[38]

↑, increase; Ag, antigen; CpG ODN, oligodeoxynucleotides contained CG motifs; CT, cholera toxin; FCA, Freund's complete adjuvant; FIA, Freund's incomplete adjuvant; i.m, intramuscular; i.n, intranasal; i.p, intraperitoneally; IFN-γ, interferon-γ; IL, interleukin; NR, not reported; PLGA, polylactide-co-glycolide acid; poly (I:C), polyinosinic–polycytidylic acid; Re, Ginsenoside Re; ROP, rhoptry protein or rhoptry antigens; s.c, subcutaneous; SI, stimulation index; SIgA, secretory immunoglobulin A; *T. gondii*, *Toxoplasma gondii*; Th, T helper.

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