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Supplementary Table 1. Baseline characteristics of included studies based on immunization experiments with *Toxoplasma gondii* DNA-encoding MICs in mouse models (single antigens)

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
MIC2	Gold particles	Gene gun into abdomen	BALB/c ($H\text{-}2^d$) and C57BL/6 ($H\text{-}2^b$)	20 Cysts of the *T. gondii* Beverley strain, orally	Induce the production of specific antibodies ↑ IFN-γ	NR	BALB/c: Increased survival rate (40%, 30-day post challenge, p=0.015) C57BL/6: Increased survival rate (37.5%, 30-day post challenge, p=0.0151)	-	[1]
MIC3	pGM-CSF	i.m	CBA/J ($H\text{-}2^k$)	70 Cysts of the 76K strain, orally	Induced a strong IgG antibody response (p<0.05) ↑ Splenocyte proliferation p<0.05) ↑ IFN-γ in mice immunized with pMIC3+pGM-CSF (111.9±11 pg/mL, p<0.05) ↑ IL-2 (210±45 pg/mL, p<0.05 and 242±57 pg/mL, p<0.05 for pMIC3 and pMIC3+pGM-CSF groups, respectively) Similar levels of IL-4 and IL-10 between all groups (p>0.05)	Reduced (p<0.05) pMIC3: 58% pMIC3+ pGM-CSF: 74%	NR	This study describes the design of a potent DNA vaccine encoding the novel *T. gondii* target antigen, MIC3 protein, that elicits a strong specific immune response as well as providing effective protection against *T. gondii* infection.	[2]
	-	Footpad	Kunming	5×10^2 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response (p<0.05) ↑ $CD4^+$ and $CD8^+$ T lymphocytes (especially $CD8^+$, p<0.05) ↓ $CD4^+/CD8^+$ ratio (p<0.05)	NR	Increased survival time (p<0.05)	A potent DNA vaccine pcDNA3-MIC3 could elicit a strong specific immune response and induce effective protection against *T. gondii* challenge in Kunming mice, suggesting that MIC3 is a potential vaccine candidate against toxoplasmosis.	[3]
	-	i.m Groups: pcDNA 3-MIC3 (the conventional DNA vaccine plasmid) pSCA1-MIC3 (the suicidal DNA vaccine plasmid) pSCA1 PBS	BALB/c	1×10^4 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response (p<0.05) No significant difference in terms of IgG responses between the groups immunized with pSCA-MIC3 and pcDNA-MIC3 (p>0.05) ↑ Splenocyte proliferation p<0.05) However, the mean lymphocyte SI of the pSCA-MIC3 vaccinated group was higher than pcDNA-MIC3 vaccinated group, but this was not statistically significant (p>0.05). ↑ IFN-γ significantly (especially in the mice immunized with pSCA-MIC3) ↓ IL-4 (p<0.05) No significant difference in terms of IFN-γ and IL-4 responses between the pSCA-MIC3 group and pcDNA-MIC3 group (p>0.05)	NR	Increased survival rate (p<0.05)	The findings demonstrated that like conventional DNA vaccine pcDNA-MIC3, suicidal DNA vaccine pSCA-MIC3 also provided favorable efficacy, but it could improve the biosafety of conventional vaccines. This result suggested that suicidal DNA vaccine pSCA-MIC3 is a potential candidate vaccine against toxoplasmosis.	[4]

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Supplementary Table 1. Continued

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
	-	i.m	BALB/c	1×10^3 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response ($p<0.01$) ↑ Splenocyte proliferation ($p<0.05$) ↑ IFN-γ ($p<0.05$) Similar levels of IL-4 and IL-10 between the different immunized and unimmunized groups ($p>0.05$)	NR	Prolonged survival time ($p<0.05$)	-	[5]
	-	i.m	ICR	1×10^3 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response with predominance of IgG2a over IgG1 ($p<0.05$) ↑ Splenocyte proliferation ($p<0.05$) ↑ IFN-γ (346 ± 31 pg/mL, $p<0.05$) and IL-4 (51 ± 11 pg/mL, $p<0.05$)	NR	Prolonged survival time (14 days compared with 7 days in control, $p<0.05$)	Our study indicates that the introduction of multi-antigenic DNA vaccine is more powerful and efficient than single-gene vaccine.	[6]
	-	i.m	BALB/c	1×10^4 Tachyzoites, RH strain, i.p	↑ Levels of IgG1 and IgG2a ($p<0.05$) ↑ IFN-γ and IL-4 ($p<0.05$) ↑ Proliferation SI ($p<0.05$)	NR	Prolonged survival time (13 days compared with 6 days in control, $p<0.05$)	These results indicated DNA vaccine encoded MIC3 gene of *T. gondii* capable to induced partially protection against toxoplasmosis.	[7]
	-	i.m	BALB/c	1×10^2 Tachyzoites, RH strain, i.p	↑ Levels of IgG antibodies ($p<0.05$)	NR	Prolonged survival time (11 days compared with 7 days in control)	These results demonstrate that TgMIC3 could elicit some protection against toxoplasmosis.	[8]
	-	i.m	BALB/c	1×10^4 (high dose) and 1×10^2 (low dose) tachyzoites, RH strain, i.p	↑ Specific IgG antibody response ($p<0.05$) ↑ IFN-γ ($p<0.05$)	↓ Parasite burden in brain and liver ($p<0.01$)	High dose: increased survival time (14 days compared with 6-7 days in controls, $p<0.05$) Low dose: increased survival rate (60%, 32 days after challenge, $p<0.05$)	The present study indicates that MIC3 showed the potential as target for vaccine investigation against toxoplasmosis.	[9]
MIC4	-	i.m	BALB/c	1×10^3 Tachyzoites, RH strain, i.p	Induced a strong IgG and IgA antibodies responses The predominance of IgG2a over IgG1 ↑ IFN-γ (632 ± 96 pg/mL, $p<0.05$) and IL-12 (415 ± 23 pg/mL, $p<0.05$)	NR	Prolonged survival time in mice (death within 11 days, $p<0.05$) All mice in the control groups died within 6-7 days.	Although this vaccine elicited humoral and cellular immune response and prolonged the life of mice which infected with the RH tachyzoites, they can't protect mice from death or unhealthy. So it is a long way for us to explore an authentic vaccine against this parasite.	[10]
	-	i.m	Kunming	1×10^3 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response ($p<0.05$) ↑ IFN-γ (657 ± 32.74 pg/mL, $p<0.05$), IL-2 (614.33 ± 30.92 pg/mL, $p<0.05$), IL-4 (281.33 ± 14.29 pg/mL, $p<0.05$) and IL-10 (608.33 ± 17.01 pg/mL, $p<0.05$) ↑ Proliferation SI (1.61 ± 0.05, $p<0.05$)	NR	Increased survival time ($p<0.05$)	The results showed that *T. gondii* MIC4 is a potential vaccine candidate against toxoplasmosis.	[11]

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Supplementary Table 1. Continued

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
MIC6	-	i.m	Kunming	1×10^3 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response ($p<0.05$) ↑ Proliferation SI (1.50±0.07, $p<0.05$) ↑ IFN-γ (557.67±28.04 pg/mL, $p<0.05$), IL-2 (522.33±66.53 pg/mL, $p<0.05$), IL-4 (202.67±14.74 pg/mL, $p<0.05$) and IL-10 (523±37.36 pg/mL, $p<0.05$)	NR	Prolonged survival time in mice (13.3±1.2 days, $p<0.05$) Control mice were died within 5 days.	Our data demonstrate, for the first time, that MIC6 triggered a strong humoral and cellular response against *T. gondii*, and that the Ag is a potential vaccine candidate against toxoplasmosis, worth further development.	[12]
	-	i.m	Kunming	20 and 80 cysts of strain PRU, i.g	Induced a strong IgG antibody response ($p<0.05$) ↑ Proliferation SI (4.29±0.18, $p<0.05$) ↑ Proliferation SI (4.29±0.18, $p<0.05$) ↑ IFN-γ (475.8±21.2 pg/mL, $p<0.05$), IL-2 (208.0±7.2 pg/mL, $p<0.05$), IL-12 (130.5±7.51 pg/mL, $p<0.05$), IL-4 (115.5±7.9 pg/mL, $p<0.05$) and IL-10 (68.3±1.9 pg/mL, $p<0.05$)	Reduced (39.81%, $p<0.05$)	Prolonged survival time ($p<0.05$) Control mice were died within 25 days.	Immunization with the recombinant plasmid DNA encoding *T. gondii* MIC6 offers protective efficacy, and this is a promising vaccine candidate against chronic toxoplasmosis.	[13]
MIC8	-	i.m	Kunming	1×10^3 t, RH strain, i.p	Induced a strong IgG antibody response ($p<0.05$) ↑ Proliferation SI (1.39±0.13, $p<0.05$) ↑ IFN-γ (484.67±25.58 pg/mL, $p<0.05$), IL-2 (359.33±61.76 pg/mL, $p<0.05$), IL-4 (189.00±18.33 pg/mL, $p<0.05$) and IL-10 (404.33±67.87 pg/mL, $p<0.05$)	NR	Increased survival time (10.3±0.9 days, $p<0.05$) Control mice were died within 5 days.	These data demonstrate that the *T. gondii* MIC8 is a potential vaccine candidate against toxoplasmosis.	[14]
	mIL-15 and mIL-21	i.m	Kunming	Acute: 1×10^3 tachyzoites, RH strain, i.p and 80 cysts PRU strain, orally Chronic: 20 cysts PRU strain, orally	Induced a strong IgG antibody response ($p<0.05$) Increased both IgG1 and IgG2a with the predominance of IgG2a over IgG1 ↑ Proliferation SI (2.37±0.14, $p<0.05$) ↑ IFN-γ (808.84±46.42 pg/mL, $p<0.05$), IL-2 (495.73±45.81 pg/mL, $p<0.05$), IL-12 p-70 (317.08±37.41 pg/mL, $p<0.05$), IL-4 (168.78±22.64 pg/mL, $p<0.05$), and IL-10 (151.75±28.28 pg/mL, $p<0.05$) ↑ Percentages of $CD4^+$ T and $CD8^+$ cells ($p<0.05$)	Reduced (63.82%, $p<0.05$)	Increased survival time 1×10^3 tachyzoites, RH strain, i.p: 16.2±1.30 days, $p<0.05$ 80 Cysts PRU strain, orally: 44.8±4.45 days, $p<0.05$	The present study demonstrates, for the first time, a synergistic effect of rIL-15 and rIL-21 genes in augmenting the efficacy of TgMIC8 DNA vaccine through induction of strong humoral and cellular immune responses which were protective against *T. gondii* challenge.	[15]
MIC11	-	i.m	BALB/c	1×10^3 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response ($p<0.05$) ↑ Splenocyte proliferation ($p<0.05$) ↑ IFN-γ, IL-2, and IL-12 ($p<0.05$) Similar levels of IL-4 between mice vaccinated with pcDNA/MIC11and control groups ($p>0.05$)	NR	Increased survival rate (17%, 15-day post challenge, $p<0.05$) Control mice were died within 8-10 days.	These data suggest that *T. gondii* MIC11 is a reasonable vaccine candidate deserving further studies, and pcDNA/MIC11 is a potential strategy for the control of toxoplasmosis.	[16]
MIC13	-	i.m	Kunming	Acute: 1×10^3 tachyzoites, RH strain, i.p Chronic: 10 tissue cysts PRU strain, i.g	↑ IgG antibodies ($p<0.05$) ↑ Proliferation SI (1.74±0.05, $p<0.05$) ↑ IFN-γ (342±20.2 pg/mL, $p<0.05$), IL-2 (228.3±15 pg/mL, $p<0.05$), IL-4 (185±8.2 pg/mL, $p<0.05$) and IL-10 (358.3±33.8 pg/mL, $p<0.05$)	Reduced (57.14%, $p<0.05$)	Increased survival time (21.3±11.3 days, $p<0.05$) Control mice were died within 10 days.	*T. gondii* MIC13 is a potential vaccine candidate, worth being included in future vaccine development against acute and chronic *T. gondii* infection.	[17]

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Supplementary Table 1. Continued

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
PLP1	pIL-18	i.m	Kunming	1×10^3 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response ($p<0.05$) ↑ Proliferation SI (4.51±0.68 and 7.95±0.87 in mice immunized with pVAX-TgPLP1 and pVAX-TgPLP1+pVAX-IL-18, respectively, $p<0.05$) ↑ IFN-γ, IL-2, IL-4, and IL-10 ($p<0.05$)	NR	Increased survival time pVAX-TgPLP1: 11.3±0.9 days, $p<0.05$ pVAX-TgPLP1+ pVAX-IL-18: 12.7±1.2 days, $p<0.05$	This study demonstrated, for the first time, that TgPLP1 induced a strong protective humoral and cellular response against *T. gondii*, indicating that it is a potential vaccine candidate against toxoplasmosis, worth further development. The murine IL-18 enhanced such immune protection. Further studies are warranted to evaluate the immune efficacy of this DNA vaccine construct in other animal host species against toxoplasmosis.	[18]
	-	i.m	Kunming	20 and 80 cysts of strain PRU, i.g	Induced a strong IgG antibody response ($p<0.05$) ↑ Proliferation SI (4.20±0.27, $p<0.05$) ↑ IFN-γ (471.5±28.9 pg/mL, $p<0.05$), IL-2 (206.3±28.2 pg/mL, $p<0.05$), IL-12 (130.3±17.7 pg/mL, $p<0.05$), IL-4 (118.5±6.4 pg/mL, $p<0.05$), and IL-10 (67.3±2.9 pg/mL, $p<0.05$)	Reduced (43.99%, $p<0.05$)	Increased survival time ($p<0.05$)	Immunization with the recombinant plasmid DNA encoding *T. gondii* TgPLP1 offers protective efficacy, and this is a promising vaccine candidate against chronic toxoplasmosis.	[13]
M2AP	Gold particles	Gene gun into abdomen	BALB/c ($H\text{-}2^d$) and C57BL/6 ($H\text{-}2^b$)	20 Cysts of the *T. gondii* Beverley strain, orally	Induce the production of specific antibodies ↑ IFN-γ	NR	BALB/c: increased survival rate (20%, 30-day post challenge, none-significant) C57BL/6: none of the mice from pM2AP, or control groups survived the infection.	-	[1]
AMA1	Gold particles	Gene gun into abdomen	BALB/c ($H\text{-}2^d$) and C57BL/6 ($H\text{-}2^b$)	20 Cysts of the *T. gondii* Beverley strain, orally	Induce the production of specific antibodies ↑ IFN-γ	NR	BALB/c: increased survival rate (60%, 30-day post challenge, p=0.0058) C57BL/6: increased survival rate (37.5%, 30-day post challenge, p=0.0038)	The AMA1 gene appears to generate a strong specific immune response and also provides efective protection against toxoplasmosis.	[1]
SPATR	-	i.m	BALB/c	1×10^2 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response ($p<0.05$) ↑ Both IgG1 and IgG2a with the predominance of IgG2a over IgG1 ($p<0.05$) ↑ Proliferation SI (1.24±0.14, $p<0.05$) Elicited both Th1/Th2 type response ↑ IFN-γ (672.87±8.35 pg/mL, $p<0.05$), IL-2 (367.93±10.30 pg/mL, $p<0.05$), IL-4 (212±7.42 pg/mL, $p<0.05$) and IL-10 (261.8±10.03 pg/mL, $p<0.05$)	NR	Increased survival time (15.7±1.42 days, $p<0.05$) Control mice were died within 7 days.	The current study indicated that pVAX1-TgSPATR induce a *T. gondii* specific immune response and might be a promising vaccine candidate against toxoplasmosis. To the best of our knowledge, this is the first report to evaluate the immunoprotective value of TgSPATR against *T. gondii*.	[19]

MIC, microneme proteins; IFN-γ, interferon-γ; NR, not reported; pGM-CSF, plasmid encoding granulocyte-macrophage colony-stimulating factor; i.m, intramuscular; IL, interleukin; PBS, phosphate-buffered saline; i.p, intraperitoneal; i.n, intranasal; SI, stimulation index.

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