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Supplementary Table 2. Baseline characteristics of included studies based on immunization experiments with *Toxoplasma gondii* DNA-encoding MICs in mouse models (mixed antigens)

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
MIC2 (MIC2a and MIC2b) +MIC3+MIC4+ M2AP+AMA1	-	Plasmid, i.m	BALB/c	30 Cysts of avirulent *T. gondii* strain SSI119, orally	*T. gondii*–specific IgG against MIC2a, MIC4, M2AP, and AMA1 protein products, whereas no IgG response was detected against MIC2b and MIC3.	Reduced (84%, $p<0.0021$)	NR	The results showed that a combination of these antigenic regions should be considered in the design of potential vaccines against toxoplasmosis.	[20]
MIC3+SAG1	-	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 ($p<0.05$)	These results indicated that cocktail-vaccine immunization could be employed as an alternative way to develop new-generation vaccines against *T. gondii* infection.	[5]
MIC3+ROP18	-	i.m	ICR	1×10^3 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response ($p<0.05$) Predominance of IgG2a over IgG1 (IgG2a values in the pMIC3-ROP18 immunized group were significantly higher than the single-gene immunized group, $p<0.05$) ↑ Splenocyte proliferation $p<0.05$) ↑ IFN-γ (849 ± 86 pg/mL, $p<0.05$) and IL-4 (66 ± 14 pg/mL, $p<0.05$) Higher levels of IFN-γ in mice vaccinated with pMIC3-ROP18, compared that other groups ($p<0.05$) No significant difference between multi-antigenic group and single-gene group in IL-4 production ($p>0.05$)	NR	Increased survival time (19 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. These results suggested that multi-antigenic DNA immunization might be an important approach to achieve an effective vaccine against *T. gondii*.	[6]
MIC3+GRA1	-	i.m	BALB/c	1×10^4 (high dose) and 1×10^2 (low dose) tachyzoites, RH strain, i.p	↑ Specific IgG antibody response ($p<0.05$) The levels of IgG in pMIC3-GRA1 group was higher than the group of pGRA1 or pMIC3 ($p<0.05$). ↑ IFN-γ ($p<0.05$) Significantly higher levels of IFN-γ production in the mice vaccinated with pMIC3-GRA1 than pGRA1 or pMIC3 ($p<0.05$)	↓ Parasite burden in brain (57.5%, $p<0.01$) and liver (55.1%, $p<0.01$)	High dose: increased survival time (15.7 ± 1.88 days, $p<0.05$) Low dose: increased survival rate (80%, 32 days after challenge, $p<0.01$)	These observations suggest that multi-antigenic DNA vaccine is more eligible for effective anti- *T. gondii* vaccine investigation. Further studies should be considered on other potent effective antigens, suitable adjuvants and other animal models.	[9]
MIC3+GRA5	-	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 (12 days compared with 6 days in control, $p<0.05$)	These results indicated DNA vaccine encoded MIC3 and GRA5 genes of *T. gondii* capable to induced partially protection against toxoplasmosis.	[7]

(*Continued to the next page*)

Supplementary Table 2. Continued

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
MIC4+SAG1	pCTA$_2$/B	i.n	BALB/c	1×10^3 Tachyzoites, RH strain, i.p	Induced a strong IgG and IgA antibodies responses ($p<0.05$) The levels of IgA and IgG antibodies were higher in the sera of mice co-immunized with pCTA$_2$/B than in the sera of mice immunized with pMIC4-SAG1 alone ($p<0.001$). The predominance of IgG2a over IgG1 IgG2a values in the pMIC4-SAG1 plus pCTA$_2$/B immunized group were significantly higher than the pMIC4-SAG1 immunized group ($p<0.01$). Similar values of IgG1 between pMIC4-SAG1 and pMIC4-SAG1 +pCTA2/B ($p>0.05$) ↑ Splenocyte proliferation ($p<0.05$) Co-administration of pCTA2/B augmented splenocyte proliferation when compared to proliferation by spleen cells from mice immunized with pMIC4-SAG1 alone ($p<0.001$) ↑ IFN-γ (1,136±152 pg/mL, $p<0.05$ and 1,874±465 pg/mL, $p<0.001$ for pMIC4-SAG1 and pMIC4-SAG1+pCTA$_2$/B groups, respectively) ↑ IL-12 (845±37 pg/mL, $p<0.05$ and 1,228±98 pg/mL, $p<0.001$ for pMIC4-SAG1 and pMIC4-SAG1+pCTA$_2$/B groups, respectively) ↓ IL-4 in mice immunized with pMIC4-SAG1+pCTA$_2$/B (23±3 pg/mL, $p<0.05$)	NR	pMIC4-SAG1: prolonged survival time (death within 14 days, $p<0.05$) pMIC4-SAG1+ pCTA2/B: increased survival rate (14%, 20-day post challenge, $p<0.05$) All mice in the control groups died within 6-7 days.	These results provided a foundation for further studies toward the use of multiantigenic DNA vaccines combined with mucosal adjuvants to develop an efficient and long-term protective immunity against *T. gondii* and other intracellular parasite infections.	[10]
MIC6+PLP1	pIL-18	i.m	Kunming	20 and 80 cysts of strain PRU, i.g	Induced a strong IgG antibody response in the sera of mice immunized with pIRESneo-MIC6-TgPLP1 or pIRESneo-MIC6-TgPLP1+pVAX-IL-18 (especially in the latter group, $p<0.01$) ↑ Proliferation SI (6.74±0.14 and 8.63±0.15 in mice immunized with pIRESneo-MIC6-TgPLP1 and pIRESneo-MIC6-TgPLP1+pVAX-IL-18, respectively, $p<0.05$) ↑ IFN-γ, IL-2, IL-12, IL-4, and IL-10 ($p<0.05$)	Reduced (61.6% and 65.43% in mice immunized with pIRESneo-MIC6-TgPLP1 and pIRESneo-MIC6-TgPLP1+ pVAX-IL-18, respectively, $p<0.05$)	Prolonged survival time pIRESneo-MIC6-TgPLP1: 42.8±2.9 days ($p<0.05$) pIRESneo-MIC6-TgPLP1+ pVAX-IL-18: 45.0±2.9 days ($p<0.05$) Control mice were died within 25 days.	Immunization with the recombinant plasmid DNA encoding *T. gondii* TgPLP1 and MIC6 offers protective efficacy, and this is a promising vaccine candidate against chronic toxoplasmosis. The application of targeted stage-specific immunization strategies and/or combination with other effective antigens should improve the protective effect of TgPLP1 or MIC6 and potentially eliminate or significantly mitigate the risks of brain cyst reactivation during chronic infection by *T. gondii*.	[13]

MIC, microneme proteins; i.m, intramuscular; IFN-γ, interferon-γ; IL, interleukin; SI, stimulation index; i.n, intranasal.

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