

Supplementary Table 5. Examples of immunization experiments with epitope-based vaccines against *T. gondii* in mouse models

Antigen	Adjuvant or carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
MEG, containing T- and B-cell epitopes SAG1 (59-67), P1 SAG1 (246-256), P2 GRA1 (176-186), P3 ROP2 (199-216), P4 GRA4 (235-245), P5 Groups:	CpG motif and CTXA ₇ /B	Plasmid, i.m	BALB/c	1 × 10 ³ Tachyzoites, i.p RH strain, i.p	↑ Antibody response (IgG) in the mice immunized with pVAX1-MEG (p= 0.009) and pVAX1-MEG-CTXA ₇ /B (p=0.006) group, compared than negative controls ↑ Serum IgA response in the mice immunized with pVAX1-MEG-CTXA ₇ /B (p<0.05, compared to other immunized group) The predominances of the levels of IgG2a over IgG1 (especially in mice immunized with pVAX1-MEG-CTXA ₇ /B) Higher IgG2a values in mice immunized with pVAX1-MEG-CTXA ₇ /B, compared with pVAX1-MEG (p<0.001) and similar values of IgG1 between these groups (p=0.834) ↑ Splenocyte proliferation significantly (especially in mice immunized with pVAX1-MEG-CTXA ₇ /B) ↑ CTL activity (especially in mice immunized with pVAX1-MEG-CTXA ₇ /B) ↑ IFN-γ and IL-2 (p<0.05)	NR	Increased survival time (p<0.05 and p<0.001) in mice immunized with pVAX1-MEG and pVAX1-MEG-CTXA2/B, respectively, compared with three control groups 20% Survival in mice immunized with pVAX1-MEG-CTXA2/B All mice in control groups died within 7 days.	This study is the first report of a multi-epitope DNA construct strategy as a potential DNA vaccine against toxoplasmosis. Furthermore, we have also demonstrated that the use of a combination of this DNA vaccine component with CpG motif and CTXA ₇ /B as genetic adjuvant enhanced both the magnitude and breadth of immune responses accompanied by significant increasing of survival rate in vaccinated mice.	[47]
MEG, containing T- and B-cell epitopes SAG1 (40-50 aa) – (236-247 aa) – (181-192 aa) GRA2 (153-169 aa) – (113-125 aa) GRA7 (162-175 aa) – (221-235 aa) – (153–162 aa) ROP16 (240-253 aa) – (364-372 aa) – (470-483 aa) – (541-549 aa) Groups:	pRANTES	Plasmid, i.m	BALB/c	1 × 10 ³ Tachyzoites, i.p RH strain, i.p	↑ Levels of IgG in mice vaccinated with pTgMEG and pTgMEG+pRANTES (especially in the latter group), compared with controls (p<0.05) The predominance of IgG2a over IgG1 (especially in the pTgMEG+pRANTES group) significantly ↑ IFN-γ in the experimental groups, compared with the control groups (p<0.05) Higher IFN-γ secretion in immunized mice with pTgMEG+pRANTES compared with pTgMEG group (p<0.05) IL-4 or IL-10 production did not differ among all groups. ↑ Percentages of CD4 ⁺ and CD8 ⁺ T cells in pTgMEG and pTgMEG+pRANTES (especially in latter group)	NR	Increased survival time (p<0.05) pTgMEG; 13 days pTgMEG+pRANTES: 17 days All control mice died within 6-7 days.	The DNA vaccine and the genetic adjuvant revealed in this study might be new candidates for further vaccine development against <i>T. gondii</i> infection, although developing an effective vaccine against <i>T. gondii</i> is not only a tedious mission but, also adifficult a challenge.	[48]

↑, increase; Ag, antigen; CTLs, cytotoxic T lymphocytes; CTXA₇/B, A₇/B subunits of cholera toxin; GRA, dense granule antigens; i.m, intramuscular; i.p, intraperitoneally; IFN-γ, interferon-γ; IL, interleukin; MEG, multi-epitope genes; NR, not reported; PBS, phosphate-buffered saline; RANTES, regulated upon activation normal T-cell expressed and secreted; ROP, rhostry protein or rhostry antigens; SAG, surface antigens; *T. gondii*, *Toxoplasma gondii*.