Supplementary Table 5. Examples of immunization with live-attenuated vectors expressing *Toxoplasma gondii* MICs in mouse models

Antigen	Adjuvant/ Carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
MIC3	-/pseudorabies virus (PRV)	i.m	BALB/c	1×10^2 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response Developed neutralizing antibodies ↑ Splenocyte proliferation ($p<0.05$) ↑ IFN-γ, IL-2, and IL-10 ($p<0.05$) Similar levels of IL-4 in all groups ($p>0.05$)	NR	Increased survival rate (50%, 28-day post challenge, $p<0.05$) Control mice were died within 9-10 days.	These results suggested that expression of protective Ag of *T. gondii* in PRV is a novel approach towards the development of a vaccine against txoplasmosis.	[23]
	-/baculovirus (bv)	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 ($p<0.05$) Control mice were died within 4 days.	These results suggest that an excellent vector-mediated vaccine strategy might be used to develop a new generation of vaccines against *T. gondii* infection.	[5]
	-/*S. typhimurium* strain SV4089 Dam– and PhoP– mutant	Orally	ICR	5×10^2 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response ($p<0.05$) ↑ Splenocyte proliferation ($p<0.05$) Mixed IgG1/IgG2a response with the predominance of IgG2a over IgG1 ($p<0.05$) ↑ IFN-γ (721 ± 142 pg/mL, $p<0.01$)	NR	Prolonged survival time (11 days compared with 6 days in control, $p<0.05$)	This study preliminarily shows that attenuated *S. typhimurium* strain (Dam– and PhoP–) could be utilized as an oral delivery vector for recombinant eukaryotic expression plasmids as DNA vaccines for prevention from toxoplasmosis.	[24]
MIC8	-/VLP recombinant baculovirus (rBV) influenza matrix protein 1 (M1)	i.m, i.n	BALB/c	1×10^5 Tachyzoites, RH strain, orally	i.n mice group showed higher levels of *T. gondii*-specific IgG antibody response compared to i.m mice group ($p<0.01$). i.n mice group showed higher levels of *T. gondii*-specific IgG1 antibody response compared to IgG2a and IgG2b antibody responses, indicating that i.n administration induced Th2-dominant responses. Higher levels of *T. gondii*-specific IgG or IgA antibodies were detected in serum or feces in i.n mice groups ($p<0.01$). Significant reduction of tachyzoites recovered from abdominal cavities in i.n mice compared to i.m as neutralizing antibody response ($p<0.01$). Higher populations of CD_4^+ and CD_8^+ T cells and germinal center B cells were found in i.n and i.m mice groups compared to non-infected naïve and non-immunized control groups. Significantly higher populations of germinal center B cells (14.4%), CD_4^+ T cells (29.84%) and CD_8^+ T cells (12.55%) were observed in i.n mice group compared to that in i.m group ($p<0.05$, $p<0.01$).	NR	Increased survival rate i.n group: 100%, 16-day post challenge i.m group: 60%, 16-day post challenge Control mice were died within 12 days.	Our study shows the effective protection against *T. gondii* infection provided by VLPs containing MIC8 of *T. gondii*, thus indicating a potential *T. gondii* vaccine candidate.	[25]

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Supplementary Table 5. Continued

Antigen	Adjuvant/ Carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
MIC3+SAG1	-/pseudorabies virus (PRV)	i.m	BALB/c	1×10^2 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response Developed neutralizing antibodies ↑ Splenocyte proliferation ($p<0.05$) ↑ IFN-γ, IL-2, and IL-10 ($p<0.05$) Similar levels of IL-4 in all groups ($p>0.05$)	NR	Increased survival rate (66.7%, 28-day post challenge, $p<0.05$) Control mice were died within 9-10 days.	These results suggested that expression of protective antigens of *T. gondii* in PRV is a novel approach towards the development of a vaccine against txoplasmosis.	[23]
	-/baculovirus (bv)	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	Increased survival rate (50%, 22-day post challenge, $p<0.05$) Control mice were died within 4 days.	These results suggest that an excellent vector-mediated vaccine cocktail strategy might be used to develop a new generation of vaccines against *T. gondii* infection.	[5]
	-/*S. typhimurium* strain SV4089 Dam– and PhoP– mutant	Orally	ICR	5×10^2 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response ($p<0.05$) ↑ Splenocyte proliferation ($p<0.05$) Mixed IgG1/IgG2a response with the predominance of IgG2a over IgG1 ($p<0.05$) ↑ IFN-γ (1,089±163 pg/mL, $p<0.01$)	NR	Increased survival rate ($p<0.05$)	The current study shows that the oral multi-antigenic DNA vaccine, ZJ111/pSAG1-MIC3, produces partial protection against *T. gondii* challenge.	[24]
Encoding MAS and UMAS $ROP18_{347–396}$, $SAG3_{101–144}$, $MIC6_{288–347}$, $GRA7_{182–224}$, $MAG1_{58–125}$, $BAG1_{156–211}$, and $SPA_{142–200}$ DNA vaccine or/and Ad vaccine	Ubiquitin/Ad	DNA vaccines (p-MAS or p-UMAS plasmid, 100 μg each), i.m or recombinant Ad vaccine (Ad-UMAS virus, 3×10^8 PFU each), i.m or the combination of DNA vaccine (p-UMAS, 100 μg each) and recombinant Ad vaccine (Ad-UMAS virus, 3×10^8 PFU each).	BALB/c	Acute: 1×10^3 tachyzoites, RH strain (genotype I), i.p Chronic: 20 cysts PRU strain (genotype II), i.g via oral gavage	Induced a strong IgG antibody response in both p-MAS and p-UMAS immunized mice (especially in the p-UMAS group), compared to control groups. ↑ Splenocyte proliferation in both p-MAS and p-UMAS immunized mice (a further 30% increase in latter group) ↑ IFN-γ and IL-2 secretion in both p-MAS and p-UMAS immunized mice (especially in the p-UMAS group), compared to control groups ↑ Levels of an IgG1 and IgG2a in p-MAS and p-UMAS immunized mice (predominance of IgG2a over IgG1), compared to control groups ↑ Percentages of $CD4^+$ T and $CD8^+$ cells in p-MAS and p-UMAS groups Significantly higher levels of IFN-γ and IL-2 secretion and increased splenocyte proliferation in Ad-UMAS immunized mice compared with p-UMAS group ($p<0.05$) ↑ Percentages of $CD8^+$ T cells in immunized with Ad-UMAS compared with p-UMAS group ($p<0.05$)	Reduced ($p<0.01$). The brain cyst burden was 50% lower in p-MAS group (833±116), compared with the control groups p-UMAS (570±98) Ad-UMAS (469±103)	Increased survival rate p-MAS: 33% survival 28 days after challenge p-UMAS: 50% survival 28 days after challenge Control mice were died within 8-10 days.	Distinct humoral and cellular immunity induced by immunization with DNA vaccine and recombinant Ad vaccine encoding ubiquitin conjugated multistage Ag of *T. gondii*. The DNA vaccine had the advantage of inducing a stronger humoral response, whereas the Ad-vectored vaccine improved the cellular immune response.	[26]

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Supplementary Table 5. Continued

Antigen	Adjuvant/ Carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
Encoding Ad-UMAS ROP18$_{347–396}$, SAG3$_{101–144}$, MIC6$_{288–347}$, GRA7$_{182–224}$, MAG1$_{58–125}$, BAG1$_{156–211}$, and SPA$_{142–200}$	Ubiquitin/Ad	i.m, i.n, s.c, i.o, i.v	BALB/c	Acute: 1×10^3 tachyzoites, RH strain (type I), i.p Chronic: 20 cysts PRU strain (type II), i.g via oral gavage	↑ Levels of *T. gondii*-specific IgG antibodies in the five Ad-UMAS immunization routes, compared to the controls ($p<0.05$). Highest titer of IgG antibody was observed by i.m route and followed by s.c, i.n, i.o and i.v. ↑ IgG subtypes in the five Ad-UMAS immunization routes, compared to the controls ($p<0.05$) Significantly higher values of IgG2a in i.m and s.c vaccination groups, compared with other vaccination routes Significantly higher values of IgA in i.n and i.o vaccination groups, compared with other vaccination routes ↑ Percentages of $CD4^+$ and $CD8^+$ T cells in the five Ad-UMAS immunization routes, compared to the controls ($p<0.05$). Significantly higher percentages of $CD4^+$ and $CD8^+$ T cells in i.n and i.o vaccination groups, compared with other vaccination routes ↑ IFN-γ and IL-2 in the five Ad-UMAS immunization routes, compared to the controls ($p<0.05$) Significantly higher secretion of IFN-γ and IL-2 in i.n and i.o vaccination groups, compared with other vaccination routes ↑ lymphocyte proliferation ability in the five Ad-UMAS immunization routes, compared to the controls ($p<0.05$) Significantly higher lymphocyte proliferation ability in i.n and i.o vaccination groups, compared with other vaccination routes	Reduced ($p<0.05$)	Increased survival rate i.m, i.o, and i.n vaccinated groups: 50% survival rate 28 days after challenge i.v and s.c vaccinated groups: 40% survival rate 28 days after challenge. All the control mice died within 8 days.	Ad-UMAS could be an effective and safe mucosal candidate vaccine to protect animals and humans against *T. gondii* infection.	[27]

MIC, microneme proteins; i.m, intramuscular; IFN-γ, interferon-γ; IL, interleukin; i.p, intraperitoneal; i.n, intranasal; NR, Not reported; s.c, subcutaneous; i.o, intraoral; i.v, intravenous.

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