Supplementary Table 6. Examples of immunization with live-attenuated vectors expressing *T. gondii* antigens in mouse models

Antigen	Adjuvant/ Carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Refer-ence
ROP2 Groups: MVA and MVA-ROP2 viruses (10^6,10^7, and 10^8 pfu) PBS	None/MVA	i.m	Female Swiss mice	Acute: 3×10^2 tachyzoites, RH strain, i.p Chronic: 20 cysts of the ME49 strain, orally	↑ Secific IgG antibodies against the ROP2 protein Mixed Th1/Th2 immune response (predominance of IgG2a over IgG1)	Animals injected either with MVA, MVA-ROP2 or PBS showed the same results (none-significant)	Increased survival time significantly (11 days vs. 8-9 days, p=0.04)	We conclude that MVA ROP2 recombinant vaccinia virus can possibly generate an immune response, which could be useful in protection against toxoplasmosis.	[49]
ROP2 Groups: BCG/pMV262-ROP2 BCG/pMV262 PBS	None/*M. bovis* BCG	s.c	BALB/c	5×10^2 Tachyzoites, RH strain, i.p	↑ Specific immune responses against ROP2 protein in mice immunized with BCG/pMV262-ROP2 ↑ IFN-γ and IL-2 in both groups that received a single inoculation and a boost inoculation of BCG/pMV262-ROP2 ↑ Percentages of $CD4^+$ T ($p<0.01$) and $CD8^+$ cells (non-significant)	NR	Increased survival rate ($p<0.05$) Control mice were died within 8-9 days	These results indicated that *M. bovis* BCG is an adequate vector to express and present antigens of *T. gondii*, and it may be used to further study the induction of protective immunity in other animals.	[50]
MEG SAG1-$I_{59–67}$ SAG1-$II_{246–255}$ $GRA1_{176–186}$ $ROP2_{200–215}$ $GRA4_{235–243}$ $SAG2C_{36–44}$ $SAG2X_{215–223}$	$CTXA_2$/B/ *S. typhimurium* strain BRD509 $aroA^-$ and $aroD^-$ mutant	i.o, i.n and i.m	BALB/c	1×10^3 Tachyzoites, RH strain, i.p	Higher levels of IgG antibody in mice vaccinated with pVAX1-MEG-$CTXA_2$/B DNA plasmid via i.m route, compared with mice immunized with BRD509/pVAX1-MEG-CTXA2/B orally and intranasally ($p<0.05$) Higher levels of IgA antibody in mice immunized with BRD509/pVAX1-MEG-$CTXA_2$/B via i.o and i.n routes, in comparison to mice immunized intramuscularly with pVAX1-MEG-$CTXA_2$/B plasmid ($p<0.05$) ↑ Percentages of $CD8^+$ T cells in the three immunization routes, compared to the controls (the highest percentages were seen in mice vaccinated i.o with BRD509/pVAX1-MEG-$CTXA_2$/B) ↑ IFN-γ and IL-2 in mice vaccinated with pVAX1-MEG- $CTXA_2$/B, compared with the control groups ($p<0.05$) Significantly higher secretion of IFN-γ and IL-2 in the mice via i.n and i.o vaccinated with BRD509/pVAX1-MEG-$CTXA_2$/B, compared with i.m vaccination route (p=0.02) Similar values of IL-4 and IL-5 between vaccinated groups and control groups ($p>0.05$) Higher Ag specific lymphocyte proliferation activity in BRD509/pVAX1-MEG-$CTXA_2$/B i.n and i.o immunization groups, compared with i.m vaccination route with pVAX1-MEG$CTXA_2$/B ($p<0.05$)	NR	Increased survival rate i.m: 20% survival rate 10 days after challenge (pVAX1-MEG-$CTXA_2$/B) Control mice were died within 4-5 days (saline and pVAX1) i.n: 40% survival rate 10 days after challenge (BRD509/ pVAX1-MEG-$CTXA_2$/ B) Control mice were died within 5-7 days (BRD509 and BRD509/pVAX1) i.o: 60% survival rate 10 days after challenge (BRD509/ pVAX1-MEG-$CTXA_2$/ B) Control mice were died within 4-8 days (BRD509 and BRD509/pVAX1)	The results from this study indicate that a DNA vaccine encoding multi-epitopes of *T. gondii* delivered by attenuated *Salmonella* is promising.	[51]

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Supplementary Table 6. Continued

Antigen	Adjuvant/ Carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
ROP16 Groups: CAV-2-ROP16 CAV-2 PBS Blank control	None/CAV-2	i.m	BALB/c	1×10^3 Tachyzoites, RH strain, i.p	Induced a strong IgG antibody response in the recombinant virus CAV-2-ROP16 group, compared to other groups ($p<0.05$) ↑ Levels of a mixed IgG1 and IgG2a ($p<0.05$) with the predominance of IgG2a production Predominant Th1-type response had developed ↑ IFN-γ (791.13 ± 42.76 pg/mL, $p<0.05$), IL-2 (418.94 ± 34.43 pg/mL, $p<0.05$), and IL-4 (173.27 ± 18.93 pg/mL, $p<0.05$) ↑ IFN-γ and TNF-α production induced by $CD4^+$ and $CD8^+$ T cells ($p<0.05$) ↑ Splenocyte proliferation in mice immunized with CAV-2-ROP16 ↑ Percentages of $CD4^+$ T and $CD8^+$ cells in CVA-2-ROP16 immunized group	NR	Increased survival rate (25% protection until 80 days after challenge, $p<0.05$) Control mice were died within 7 days	This study presents the successful use of recombinant virus CAV-2-ROP16 in vaccination protocols to protect against i.p challenge with the virulent RH strain of *T. gondii*. This system was shown to be extremely efficient in eliciting humoral and cellular immune responses that led to a significant improvement in survival time in mice.	[52]
ROP18 Groups: CAV-2-ROP18 CAV-2 PBS Blank control	None/CAV-2	i.m	Kunming	Acute: 1×10^3 tachyzoites, RH strain (genotype I), i.p Chronic: 5 cysts PRU strain (genotype II), i.g via oral gavage	Induced a strong IgG antibody response in the recombinant virus CAV-2-ROP18 group, compared to other groups ($p<0.05$) ↑ Levels of a mixed IgG1 and IgG2a ($p<0.05$) with the predominance of IgG2a production ↑ Splenocyte proliferation in mice immunized with CAV-2-ROP18 (approximately ~21-fold higher than other groups, $p<0.05$) ↑ CTL activity in mice immunized with CAV-2-ROP18 ($p<0.05$) ↑ Percentages of $CD4^+$ T and $CD8^+$ cells in CAV-2-ROP18 immunized group ↑ IFN-γ (914.26 ± 36.56 pg/mL, $p<0.05$), IL-2 (431.07 ± 28.94 pg/mL, $p<0.05$), and IL-4 (197.29 ± 29.98 pg/mL, $p<0.05$) ↑ IFN-γ and TNF-α production in both $CD4^+$ and $CD8^+$ T cell compartments	Reduced (57.3%, $p<0.05$)	Increased survival rate (40% protection until 60 days after challenge, $p<0.05$) Control mice were died within 7 days	These results demonstrate the potential use of a CAV vector harboring the ROP18 gene in the development of a vaccine against acute and chronic toxoplasmosis.	[53]

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Supplementary Table 6. Continued

Antigen	Adjuvant/ Carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Refer-ence
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 recombinantAd 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.	[54]

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Supplementary Table 6. Continued

Antigen	Adjuvant/ Carrier	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
ROP18 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	[55]

↑, increase; Ad-UMAS, adenovirus expressing ubiquitin-conjugated multistage antigen segments; Ad, adenovirus; Ag, antigen; BCG, Bacillus Calmette-Guerin; CAV-2, canine adenovirus type-2; CTLs, cytotoxic T lymphocytes; $CTXA_2$/B, A_2/B subunits of cholera toxin; GRA, dense granule antigens; i.g, intragastrically; i.m, intramuscular; i.n, intranasal; i.o, intraoral; i.p, intraperitoneally; i.v, intravenous; IFN-γ, interferon-γ; IL, interleukin; *M. bovis, Mycobacterium bovis*; MAS, multi-stage antigen segments; MEG, multi-epitope genes; MIC, microneme antigens or microneme proteins; MVA, modified vaccinia virus Ankara; NR, not reported; PBS, phosphate-buffered saline; PFU, plaque-forming unit; ROP, rhoptry protein or rhoptry antigens; s.c, subcutaneous; *S. typhimurium, Salmonella typhimurium*; SAG, surface antigens; *T. gondii, Toxoplasma gondii*; Th, T helper; TNF-α, tumor necrosis factor α; UMAS, ubiquitin-conjugated multistage antigen segments.