Supplementary Table 6. Examples of heterologous prime-boost immunization against *Toxoplasma gondii* in mouse models

Antigen/Adjuva nt	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
AMA1/Gold particles Prime/boost: pAMA1/Ad5AMA1 pAMA1/Ad5Null pNull/Ad5AMA1 pNull/Ad5Null	Gene gun into abdomen	C57BL/6	1×10^3 PLK-GFP of *T. gondii* tachyzoites, i.p	↑ Significantly IgG antibodies in pAMA1/Ad5AMA1, pAMA1/Ad5Null, and pNull/Ad5AMA1 groups, compared with those of control mice (immunized mice with pNull/Ad5Null) Mice that were primed with pAMA1 and boosted with Ad5AMA1 showed significant increase ($p<0.001$) in anti-TgAMA1 IgG when compared with those immunized with either pAMA1/Ad5Null or pNull/Ad5Null. The pAMA1/ Ad5AMA1-immunized mice had a robust IgG1, and IgG2c antibody response to the TgAMA1 compared with that of either pAMA1/Ad5Null or pNull/Ad5Null, and the robust responses were significantly enhanced ($p<0.001$) after the booster immunization. The levels of the TgAMA1-specific IgG2c antibody were higher in mice immunized with pNull/Ad5AMA1 than in those immunized with pAMA1/Ad5AMA1 ($p<0.05$) and pAMA1/Ad5Null ($p<0.001$). There was no significant difference in the IgG1:IgG2c ratio between pAMA1/Ad5AMA1 and pNull/Ad5AMA1 immunized groups. The pNull/Ad5AMA1 immunized mice had significantly higher levels of IFN-γ, as compared with the mice immunized with pAMA1/Ad5AMA1 ($p<0.01$) and pAMA1/Ad5Null ($p<0.001$). The pAMA1/Ad5AMA1 immunized mice had higher levels of IL-4 than those immunized with pNull/Ad5AMA1 ($p<0.05$) and pNull/Ad5Null ($p<0.01$).	The pAMA1/Ad5AMA1-immunized mice produced 23% fewer brain cysts than the pNull/Ad5AMA1-immunized mice (none-significant).	Increased survival rate pAMA1/Ad5AMA1: 50%, 30-day post challenge pNull/Ad5AMA1: 37.5%, 30-day post challenge pAMA1/Ad5Null: 12.5%, 30-day post challenge None of the mice immunized with pNull/Ad5Null survived	These results demonstrate that the heterologous DNA priming and recombinant adenovirus boost strategy may provide protective immunity against *T. gondii* infection.	[28]

(*Continued to the next page*)

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Supplementary Table 6. Continued

Antigen/Adjuva nt	Ag delivery	Mouse strain	Challenge	Immune responses	Brain cyst load	Survival	Conclusions or suggestions	Reference
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 adenovirus vaccine Prime/boost: DNA/DNA (p-UMAS/p-UMAS) Ad/Ad (Ad-UMAS/Ad-UMAS) DNA/Ad (p-UMAS/Ad-UMAS) Ad/DNA (Ad-UMAS/p-UMAS)	The combination of DNA vaccine (p-UMAS, 100 μg each) and recombinant adenovirus vaccine (Ad-UMAS virus, 3×10^8 PFU each), i.m	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	Highest levels of humoral antibodies and cellular immune responses were achieved in mice immunization priming with the DNA vaccine and boosting with the Ad-UMAS vaccine. Compared with p-UMAS or Ad-UMAS immunization alone, higher levels of a specific IgG (predominance of IgG2a) and higher levels of cytokines (IFN-γ and IL-2) were obtained by priming with p-UMAS and boosting with Ad-UMAS ($p<0.05$). Priming with p-UMAS and boosting with Ad-UMAS demonstrated higher proliferation activity, compared with the other immunization strategy ($p<0.05$).	Reduced ($p<0.01$) The most significant reduction of brain cyst burden was observed by the DNA prime-Ad boost approach.	Increased survival rate 67% Survival in mice vaccinated with p-UMAS prime and Ad-UMAS boost 28 days after challenge Control mice were died within 8-10 days.	Priming vaccination with DNA vaccine and boosting with the recombinant Ad vaccine encoding ubiquitin conjugated multi-stage antigens of *T. gondii* was proved to be a potential strategy against the infection of type I and type II parasite.	[26]
rTgMIC3/FCA+pcDNA-MIC3+rTgMIC3/FIA	i.m	BALB/c	1×10^2 tachyzoites, RH strain, i.p	↑ Levels of IgG antibodies ($p<0.05$)	NR	Prolonged survival time (10 days compared with 7 days in control)	These results demonstrate that TgMIC3 could elicit some protection against toxoplasmosis.	[8]

i.p, intraperitoneal; IFN-γ, interferon-γ; IL, interleukin; NR, Not reported.

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