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

<table>
<thead>
<tr>
<th>Antigen/Adjuvant</th>
<th>Ag delivery</th>
<th>Mouse strain</th>
<th>Challenge</th>
<th>Immune responses</th>
<th>Brain cyst load</th>
<th>Survival</th>
<th>Conclusions or suggestions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMA1/Gold particles Prime/boost: pAMA1/Ad5AMA1 pAMA1/Ad5Null pNull/Ad5AMA1 pNull/Ad5Null</td>
<td>Gene gun into abdomen</td>
<td>C57BL/6 1×10^6 R.K-GFP of <em>T. gondii</em> tachyzoites, i.p.</td>
<td></td>
<td>Significantly IgG antibodies in pAMA1/Ad5AMA1, pAMA1/Ad5Null, and pNull/Ad5AMA1 groups, compared with those of control mice (immunized mice with pNull/Ad5Null)</td>
<td>The pAMA1/Ad5AMA1-immunized mice produced 23% fewer brain cysts than the pNull/Ad5AMA1-immunized mice (none-significant).</td>
<td>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</td>
<td>These results demonstrate that the heterologous DNA priming and recombinant adenovirus boost strategy may provide protective immunity against <em>T. gondii</em> infection.</td>
<td>[28]</td>
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</table>

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 pAMA1/Ad5AMA1-immunized mice produced significantly higher levels of IFN-γ, as compared with the mice immunized with pAMA1/Ad5Null (p < 0.001) 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 IL-4 than those 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).

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<tbody>
<tr>
<td>Encoding MAS and UMAS</td>
<td>The combination of DNA vaccine (p-UMAS, 100 μg each) and recombinant adenovirus vaccine (Ad-UMAS virus, 3 × 10^9 PFU each) i.m</td>
<td>BALB/c</td>
<td>Acute: 1 × 10^6 tachyzoites, RH strain (genotype II), i.p</td>
<td>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 &lt; 0.05). Priming with p-UMAS and boosting with Ad-UMAS demonstrated higher proliferation activity, compared with the other immunization strategy (p &lt; 0.05).</td>
<td>Reduced (p &lt; 0.01)</td>
<td>67% Survival in mice vaccinated with p-UMAS prime and Ad-UMAS boost 28 days after challenge</td>
<td>Increased survival rate 67% Survival in mice vaccinated with p-UMAS prime and Ad-UMAS boost 28 days after challenge</td>
<td>[26]</td>
</tr>
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<td>DNA vaccine or adenovirus vaccine</td>
<td>DNA/DNA (p-UMAS/p-UMAS)</td>
<td>BALB/c</td>
<td>PRU strain (genotype III), i.v.</td>
<td>Levels of IgG antibodies (p &lt; 0.05)</td>
<td>NR</td>
<td>Prolonged survival time (10 days compared with 7 days in control)</td>
<td>These results demonstrate that TgMIC3 could elicit some protection against toxoplasmosis.</td>
<td>[8]</td>
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<tr>
<td>Prime/boost:</td>
<td>DNA/DNA (p-UMAS/p-UMAS)</td>
<td>BALB/c</td>
<td>Chronic: 20 cysts PRU strain (genotype III), i.g via oral gavage</td>
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<tr>
<td>Ad/Ad (Ad-UMAS/Ad-UMAS)</td>
<td>DNA/Ad (p-UMAS/Ad-UMAS)</td>
<td>BALB/c</td>
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Notes: tachyzoites, RH strain, i.p; Reduced (p < 0.01).
References


15. Li ZY, Chen J, Petersen E, et al. Synergy of mIL-21 and mIL-15 in enhancing DNA vaccine efficacy against acute and chronic Toxoplasma gondii infection in mice. Vaccine 2014;32:3058-65.


