Enhanced vaccine and anti-tumor immunity using GITR ligand (GITRL) or extracellular ATP (ATPe)
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ABSTRACT
Background: GITR signaling obviates immunosuppression by CD4+CD25+Foxp3+ Tregs. The natural ligand of GITR is GITRL, a TNF superfamily ligand. Extracellular ATP (ATPe) downregulates Tregs by two mechanisms: it activates macrophages and DCs to secrete IL-10, an anti-Treg cytokine; and it is selectively toxic to Tregs. Methods: DNA vaccines encoding either HIV-1 Gag or the MSP-1 19kDa blood stage antigen of Plasmodium yoelii were studied in mice. Plasmids for novel 4-trimer secretory forms of GITRL or CD40L were added as adjuvants. After the vaccination, the mice were either analyzed for T cell and antibody responses or challenged with P. yoelii-paralyzed red blood cells to induce malaria. For tumor immunotherapy studies, established A20 lymphoma or B16F10 melanoma tumors were injected intratumorally with plasmid DNA encoding 4-trimer soluble GITRL or CD40L. In some experiments, the tumors were also injected with CpG (TLR9) and poly(I:C) (TLR3) with or without ATPe. Results: As an adjuvant, GITRL was about half as effective as CD40L in promoting CD+ T cell responses. However, GITRL was stronger than CD40L at enhancing CD4+ T cell proliferation and antibody responses to the vaccine antigen. As a malaria vaccine adjuvant, GITRL led to strong protection from malaria-induced death in mice. When used for tumor immunotherapy, injections either of GITRL or CD40L cured A20 lymphoma tumors. For B16F10 melanoma tumors, cure required the quadruple combination of CD40L + CpG + poly(I:C) + ATPe. Conclusions: 4-trimer soluble GITRL and CD40L are strongly active and can be used as vaccine adjuvants and for tumor immunotherapy.

CD40 – TLR - INFLAMMASOME SYNERGY CURES ESTABLISHED MELANOMA TUMORS

DNA VACCINE METHODS
Antigen plasmids: The antigen plasmids encoded secreted, codon-optimized forms of either HIV-1 Gag (pScGag) or the MSP1 19kDa malaria antigen from Plasmodium yoelii. TNFSF ligands: pSP-D-CD40L and pSP-D-GITRL encoded 4-trimer soluble CD40L and GITRL respectively. pmEmCD40L encoded full-length, transmembrane CD40L. Mouse vaccinations: BALB/c mice were injected i.m. in both quadriceps every other week X 5 with 80 µg of either pScGag or pMSP1 plus 4-trimer TNFSF plasmid (20 µg of either pSP-D-GITRL, pSP-D-CD40L, or control empty vector).

Immunosassays: Two weeks after the last vaccination, splenic CTLs were analyzed by IFN-γ ELISPOT using P815 stimulator cells pulsed with the H-2Kd peptide, AMQMLKETI. Malaria challenge: BALB/c mice were infected i.p. with 2 X 10^9 P. yoelii-infected RBCs.

CONCLUSIONS
► Multimeric soluble TNFSFs can be produced as highly active proteins by fusing them with the body of surfactant protein D (SP-D, Fig. 1).
► pSP-D-GITRL enhanced DNA vaccines for CD8+ T cell (Fig. 2), CD4+ T cell (Fig. 3), and Ab responses (Fig. 3). pSP-D-GITRL admixed with a malaria vaccine against malaria, a disease with a strong Treg component (Fig. 4).
► pSP-D-GITRL injections into A20 lymphoma, a Treg-rich tumor, led to long-term, tumor-free survival (Fig. 5).

SP-D-GITRL IS BETTER THAN SP-D-CD40L FOR ELICITING CD4+ T CELL AND ANTIBODY RESPONSES

Figure 1. Construction of 4-trimer, multimeric soluble GITRL and CD40L. Plasmids were constructed in the pcDNA3.1, pVAX1, or pCAGEN (similar to pCAGGS) expression vectors.

Figure 3. Plasmid DNA for 4-trimer soluble GITRL, but not CD40L, enhanced CD4+ T cell and antibody responses to vaccination. Mice were vaccinated as in Fig. 2. Left Panel: Proliferation responses to Gag protein were expressed as the stimulation index. Right Panel: Serum IgG responses were measured by ELISA on Gag protein-coated plates.

Figure 4. Plasmid DNA for 4-trimer soluble GITRL was an effective adjuvant for a malaria vaccine. pSP-D-GITRL combined with pMSP1 antigen plasmid elicited an enhanced anti-MSP1 antibody response (not shown). Upon challenge with merozoite-infected RBCs, there was significantly greater protection in mice vaccinated with pMSP1 + pSP-D-GITRL compared to pMSP1 alone (p = 0.0472). CD40L, which has been reported to contribute to malaria-induced pathology, led to more rapid death in this experimental system.

Figure 5. Intratumoral injections of either 4-trimer soluble GITRL or CD40L cure mice of established lymphoma. Mice with A20 B lymphoma tumors > 4 mm in diameter were injected intratumorally with pSP-D-GITRL or pSP-D-CD40L every other day X 5, resulting in long-term tumor-free survival. In contrast, transmembrane CD40L, pMemCD40L, had no effect.


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