CD40L multi-trimer antigen fusion protein (FortiVac™) as a vaccine design for CD8+ T cell responses.

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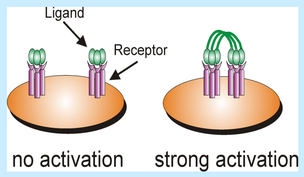
Abstract

Dendritic cells (DCs) can take up antigen via a number of routes. Of these, several studies have shown antigen uptake via the CD40 receptor generates some of the strongest CD8+ T cell responses. At the same time, stimulation through CD40 activates DCs for cross-presentation and heightened antigen presentation. Consequently, it is desirable to combine the CD40 targeting of antigen and a CD40 stimulant into a single vaccine formulation. When the CD40 stimulant is CD40 ligand (CD40L, CD154), it is important to use a multi-trimer complex that clusters CD40 in the DC membrane, thereby engaging downstream signaling pathways. Such a multi-trimer form of CD40L can be provided by fusing the extracellular domain of CD40L with the body of surfactant protein D (SPD), a self-assembling 12-chain soluble molecule with 4 trimeric "arms." When an antigen sequence is included within the SPD arm, the entire protein complex is termed "FortiVac". Studies in mice have shown that FortiVac elicits very high levels of antigen-specific CD8+ T cells with high TCR avidity and functional activity. FortiVac can be effectively delivered as a DNA vaccine i.m. and even more effectively using an adenoviral (Ad5) vector. For a FortiVac encoding HIV-1 Gag, vaccination with Ad5-FortiVac-Gag led to complete protection ("sterilizing immunity") from Vaccinia-Gag challenge (i.e., 7 log reduction of tissue virus). Thus far, FortiVac formulations have been made for HIV-1 Gag, Ebola, malaria, hepatitis B virus (HBV), and tumor-specific neoantigens. In all of these cases, there may be a clinical benefit for a vaccine that elicits very strong CD8+ T cell responses.

Receptor clustering is needed for CD40 activation

Fig. 1. CD40 receptors are activated by clustering. A stimulated CD4+ T cell expressing trimers of CD40L on its surface can form a synapse with DCs to cluster and thereby activate CD40 receptors. This leads to increased APC function and the release of cytokines and chemokines.



4-trimer form of CD40L made as a SPD fusion protein

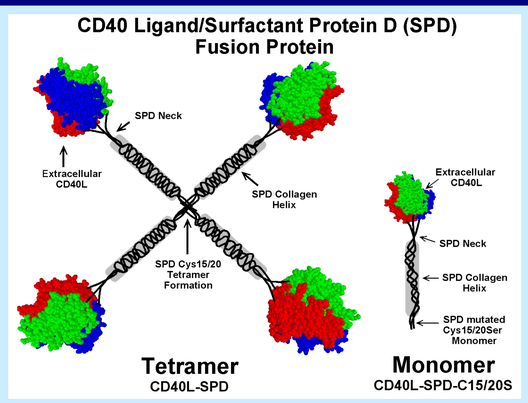


Fig. 2. SPD-CD40L. The CD40L extracellular domain (ECD) was genetically fused to surfactant protein D (SPD) as a multimerization scaffold, thereby creating a 4-trimer soluble form of CD40L capable of clustering CD40 and activating DCs.

FortiVac protein design

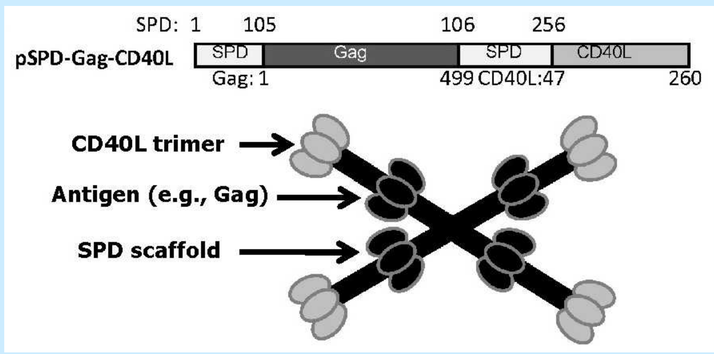


Fig. 3. SPD-Antigen-CD40L encodes a self-assembling multi-trimer form of soluble CD40L that targets antigen to CD40-bearing APCs and activates APCs at the same time. As shown, the Gag protein of HIV-1 was the initial antigen tested.

As a DNA vaccine, FortiVac-Gag elicited a huge CD8+ T cell response

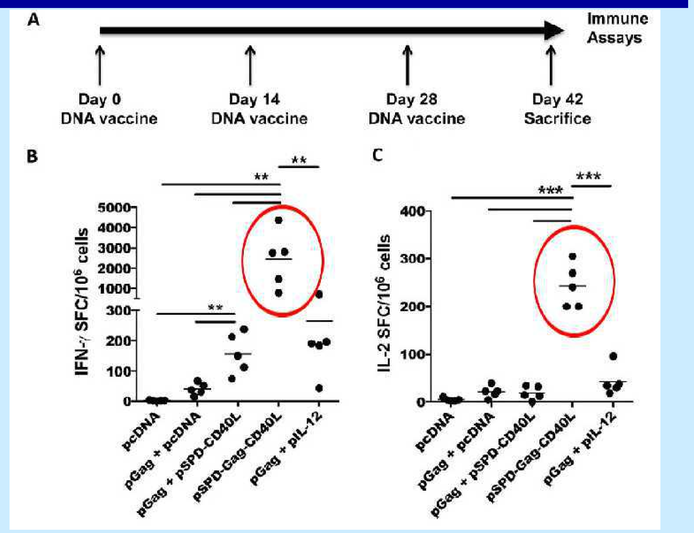


Fig. 4. FortiVac-Gag plasmid DNA injected i.m. in mice induced high-level CD8+ T cell responses. Using IFN-γ ELISPOT for a single peptide epitope in Gag, FortiVac-Gag elicited a huge increase in specific CD8+ T cells.

FortiVac-Gag elicited CD8+ T cells with high TCR avidity

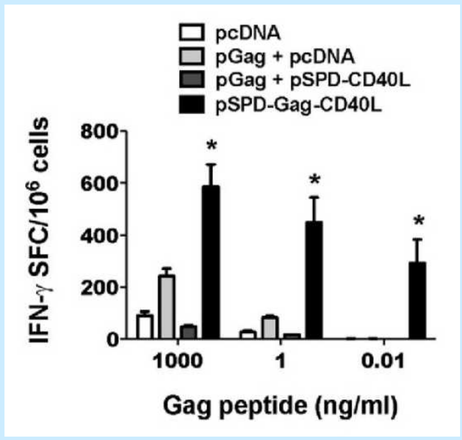


Fig. 5 TCR avidity measured by IFN-γ ELISPOT. CD8+ T cells from FortiVac-Gag vaccinated mice responded to 10 pg/ml Gag peptide, showing remarkable TCR avidity.

Adenovirus vectored FortiVac-Gag elicited sterilizing immunity to Vaccinia-Gag challenge

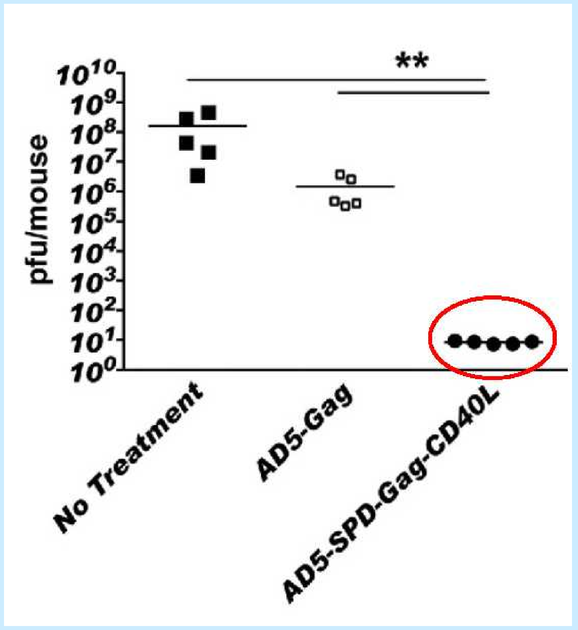


Fig. 6. Ad5-vectored FortiVac-Gag protected mice from challenge by live Vaccinia-Gag virus. Female mice were vaccinated and challenged i.p. with 10^7 PFU of Vaccinia-Gag virus. Six days later, ovaries were harvested and PFU measured to a limit of detection of 10 PFU/mouse. With the Adenovirus-delivered SPD-Antigen-CD40L vaccine, there was a **~ 7 LOG reduction in virus titer** and no virus was detected, i.e., **sterilizing immunity**.

Uses for FortiVac

(1) HIV-1 preventative and therapeutic vaccines where anti-Gag CD8+ T cells are known to help control viral replication
(2) Immuno-Oncology using shared tumor antigens like E6/E7 from HPV in cervical cancer
(3) Tumor-Specific NeoAntigen (TSNA) personalized cancer vaccines
(4) Chronic Hepatitis B Virus (HBV) infection, where anti-HBcAg-specific CD8+ T cells are known to control HBV replication
(5) Malaria where anti-CSP1 CD8+ T cells are known to control the hepatic stage of the plasmodial life cycle
(6) Universal influenza vaccine where anti-NP CD8+ T cells are known to control viral replication
(7) Ebola where anti-GP CD8+ T cells are known to protect the host

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FortiVac is protected by US patent US10072064 B2 and related counterparts worldwide.