Sterilizing antiviral CD8+ T cell immunity induced by a CD40L-based vaccine

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Abstract

CD40L engagement of the CD40 receptor on dendritic cells (DCs) is a key step in generation of CD8+ T cells. Also, targeting antigen to DCs via the CD40 receptor is an ideal way for eliciting high level CD8+ T cell responses. Consequently, we examined the vaccine effectiveness of a fusion protein comprised of multi-trimer CD40L and peptide antigen (FortiVac™).

Multi-trimer CD40L was made by fusing the extracellular domain to the body of surfactant protein D (SPD) . Placing an antigen sequence within the SPD "arms" creates FortiVac. Mice were vaccinated with either plasmid DNA vaccines or Ad5 vaccines containing FortiVac constructs. This resulted in marked CD8+ T cell responses. Using HIV-1 Gag as a model antigen, mice were completely resistant to 10^7 Vaccinia-Gag virus – i.e. sterilizing immunity.

The FortiVac is being developed for conditions where a strong CD8+ T cell response is needed - vaccines against viruses, malaria, and tumors.

Introduction

CD4+ T cells use CD40L to activate dendritic cells (DCs) to elicit CD8+ T cells

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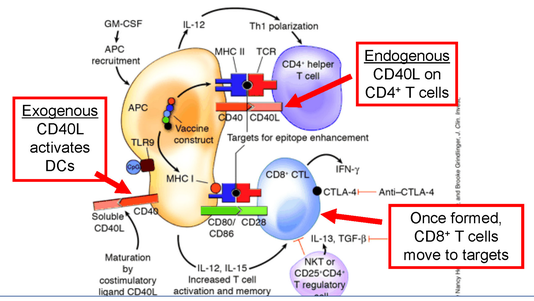

Vaccine principles supporting the FortiVac concept:

(1) Antigen targeting to CD40 is superior to other DC receptor targeting strategies for cross-presentation to CD8+ T cells.

Cohn L, et al. (Lélia Delamarre and Ira Mellman), Antigen delivery to early endosomes eliminates the superiority of human blood BDCA3+ dendritic cells at cross presentation. J Exp Med 210:1049-1063, 2013. Yin W, et al. Functional Specialty of CD40 and Dendritic Cell Surface Lectins for Exogenous Antigen Presentation to CD8(+) and CD4(+) T Cells. EBioMedicine 5:46-58, 2016.

(2) CD40 receptors on DCs are activated by SPD-Ag-CD40L In contrast, antigen targeted to DEC205 on DCs does not elicit CD8+ T cells unless a separate CD40 activator is added.

Bonifaz L, et al. Efficient targeting of protein antigen to the dendritic cell receptor DEC-205 in the steady state leads to antigen presentation on major histocompatibility complex class I products and peripheral CD8+ T cell tolerance. J Exp Med 196:1627-1638, 2002.

(3) Antigen and adjuvant delivery are synchronized when SPD-Ag-CD40L is delivered, which is important for Th1 responses.

Kamath AT, et al. Synchronization of dendritic cell activation and antigen exposure is required for the induction of Th1/Th17 responses. J Immunol 188:4828-4837, 2012.

(4) Large size of SPD-Ag-CD40L fusion protein (~100 nm) directs it to lymphatics for conveyance to APCs in draining lymph nodes.

Liu et al (Irvine group), Structure-based programming of lymph-node targeting in molecular vaccines. Nature 507:519-22, 2014.

Methods and Materials

FortiVac™ is a fusion protein of SPD, antigen, and CD40L

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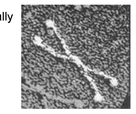
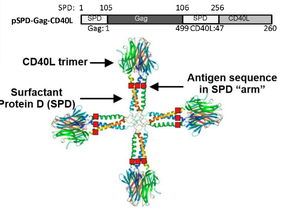
Shadow EM of naturally self-assembling 12-chain **surfactant protein D (SPD)** ~100 nm in diameter
Crouch et al., J Biol Chem 269:17311-17319, 1994



Schematic of fusion protein sequence



The design starts with SPD, a naturally occurring 12-chain molecule ~100 nm in diameter. It self-assembles from a single polypeptide sequence. In the fusion protein, the CD40L extracellular domain is placed at the ends of SPD to make a molecule with four trimers of CD40L. For FortiVac, the design further adds an antigen sequence into the "arms" of SPD.
S. Gupta et al, J Virol. 88:1492-501, 2014.

Molecular model of SPD-Ag-CD40L (FortiVac)

Results

FortiVac (SPD-Ag-CD40L) elicits high level CD8+ T cell responses

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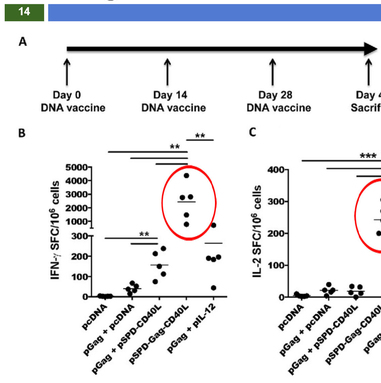

Using ELISPOT for a single peptide epitope in Gag, the SPD-Antigen-CD40L DNA vaccine produced a huge increase in specific CD8+ T cells (note the scale of the Y-axis). About 10% of these CD8+ T cells make their own IL-2, so they can essentially grow themselves without CD4+ T cell help.

S. Gupta et al, Vaccination with a fusion protein that introduces HIV-1 Gag antigen into a multi-trimer CD40L construct results in enhanced CD8+ T cell responses and protection from viral challenge by vaccinia-Gag. J Virol. 88:1492-1501, 2014.

FortiVac (SPD-Ag-CD40L) elicits high avidity CD8+ T cells

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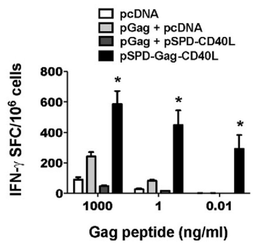

Different concentrations of Gag peptide were used in the ELISPOT assay. With spleen cells from the SPD-Antigen-CD40L vaccinated mice, almost half the CD8+ T cells were activated at 10 pg/ml (~10 pM) peptide concentration, a **remarkably strong TCR avidity**.

S. Gupta et al, Vaccination with a fusion protein that introduces HIV-1 Gag antigen into a multi-trimer CD40L construct results in enhanced CD8+ T cell responses and protection from viral challenge by vaccinia-Gag. J Virol. 88:1492-1501, 2014.

Adenovirus-delivered SPD-Ag-CD40L elicits sterilizing immunity in mice

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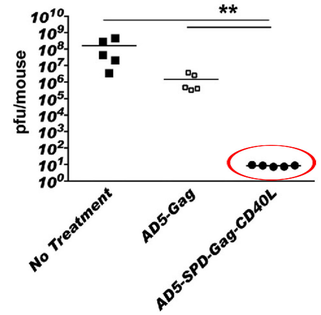

Female mice were vaccinated and challenged i.p. with 10^7 PFU of Vaccinia-Gag virus. Six days later, ovaries were harvested and PFU measured to a limit of detection of 10 PFU/mouse. With the **Adenovirus-delivered** SPD-Antigen-CD40L vaccine, there was a ~ **7 LOG reduction in virus titer** and no virus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

S. Gupta et al, Vaccination with a fusion protein that introduces HIV-1 Gag antigen into a multi-trimer CD40L construct results in enhanced CD8+ T cell responses and protection from viral challenge by vaccinia-Gag. J Virol. 2014 Feb;88(3):1492-501.

Discussion

CD8+ T cells are needed for cancer immunotherapy and for preventative vaccines against certain infections (CMV, malaria, dengue, and Ebola). In nature, the production of CD8+ T cells requires CD4+ T cells that express CD40 ligand (CD40L) which in turn activates antigen-presenting dendritic cells (DCs). Multimeric's technology bypasses the need for CD4+ T cells by fusing CD40L with surfactant protein D (SPD) to make SPD-CD40L, a highly active soluble 4-trimer protein. FortiVac™ refers to SPD-CD40L that has the antigen (Ag) placed into the SPD "arms" (SPD-Ag-CD40L). FortiVac vaccines elicit extremely high levels of CD8+ T cells with strong T cell receptor (TCR) avidity and extraordinary efficacy in vivo.

Conclusions

The production of CD8+ T cells requires CD4+ T cells that express CD40 ligand (CD40L) which in turn activates antigen-presenting dendritic cells (DCs)

Multimeric's technology bypasses the need for CD4+ T cells by fusing CD40L with surfactant protein D (SPD) to make SPD-CD40L, a highly active soluble 4-trimer protein.

FortiVac™ refers to SPD-CD40L that has the antigen (Ag) placed into the SPD "arms" (SPD-Ag-CD40L).

FortiVac vaccines elicit extremely high levels of CD8+ T cells with strong T cell receptor (TCR) avidity and extraordinary efficacy in vivo.

FortiVac vaccines are is being developed for infections where high-level CD8+ T cells are needed (e.g. viruses and malaria) and for tumors.

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References

S. Gupta et al, Vaccination with a fusion protein that introduces HIV-1 Gag antigen into a multi-trimer CD40L construct results in enhanced CD8+ T cell responses and protection from viral challenge by vaccinia-Gag. J Virol. 2014 Feb;88(3):1492-501.

GSW and RSK are inventors on worldwide patents including US 10,072,064 B2 and US 10,774,149 B2.