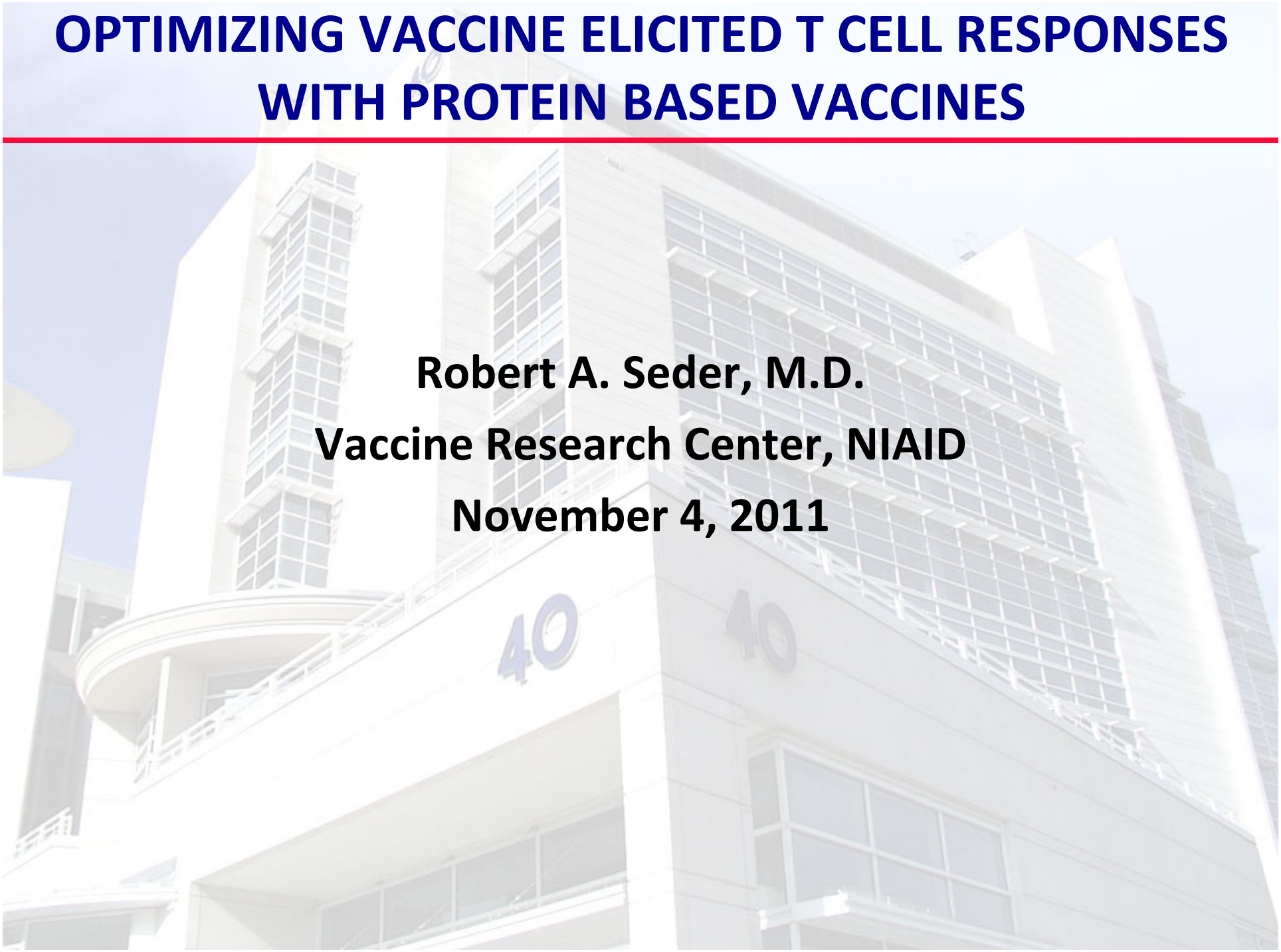
OPTIMIZING VACCINE ELICITED T CELL RESPONSES WITH PROTEIN BASED VACCINES



Robert A. Seder, M.D.

Vaccine Research Center, NIAID

November 4, 2011

Vaccines Against HIV, Malaria and Tuberculosis Will Require Antibody and/or Cell-Mediated Immunity

- **Design vaccines that elicit broad-based immunity**
- **Define antibody and T cell correlates of protection**

Tool Box of Vaccine Vectors in Current Clinical Studies for HIV, Malaria and Tuberculosis

- DNA
- Adenovirus (Ad5, Ad26, Ad35, Chimp)
- Poxvirus (MVA, NYVAC, Alvac)
- Protein/Adjuvant

Focus of this presentation:

- Formulation and delivery of proteins to DCs are critical for optimizing T cell immunity

- "Prime-boost immunization" with protein and viral vaccines improve T cell immunity

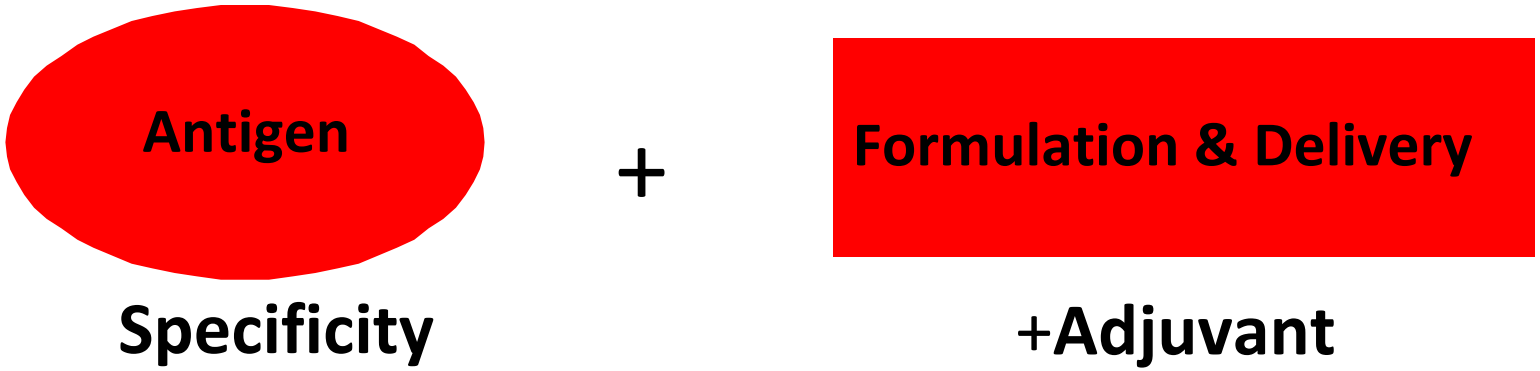
Rationale for Protein Based Vaccines

1. **Protein vaccines can induce broad-based immune responses**
   - **Antibody**
   - **Th1 *and* CD8+ T cell responses**

2. **Protein based vaccines can be used in prime-boost regimens**

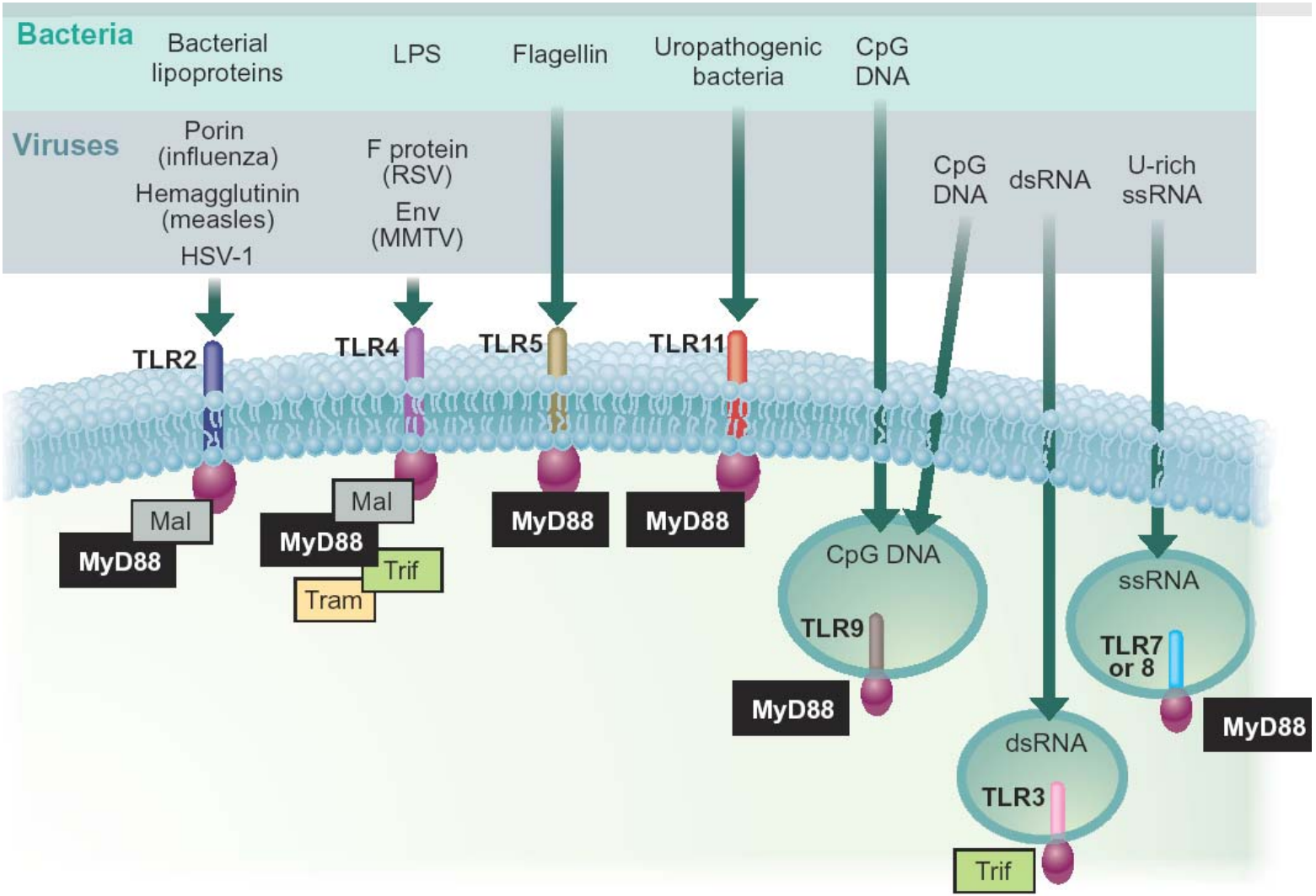
1. **Protein vaccines are not limited by pre-existing immunity**

Optimizing T Cell Responses With Protein Vaccines Require Formulation and Adjuvants



- **Vehicle**-Oil/water, Alum, Liposomes, ISCOMS, Nanoparticles
- **Conjugation-**Physically couple protein to the adjuvant (TLR ligand)
- **Targeting**-Protein linked to antibody specific to dendritic cells

Toll-like Receptors Recognize Conserved Microbial Structures



Adjuvants:

TLR Ligands Activate Distinct Human Dendritic Cell Subsets

CD141/BDCA-3 DCs

Conventional DCs

Plasmacytoid DCs

Cytokines:	IL-12/IFNβ	IL-12	Type I IFN
Ag Presentation:	CD4/CD8	CD4/CD8	-

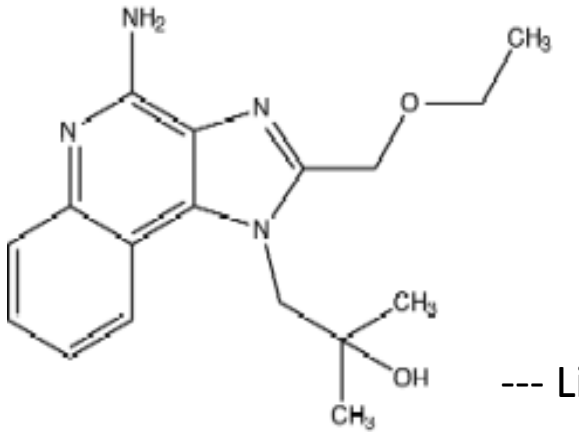
TLR Expression				TLR Ligand
TLR 4	-	+	-	LPS (MPL)
TLR 3	+	+	-	dsRNA (Poly IC)*
TLR 7	-	-	+	ssRNA (Imiquimod)
TLR 8	+	+	-	(R-848)
TLR 9	-	-	+	CpG DNA

*Poly I:C can induce IFN-α via non-TLR independent pathways (MDA-5)

Formulation

1. TLR7 and 8 agonists (imidazoquinoline) are small synthetic molecules
   - Potent inducer of innate cytokines (IL-12 and Type I IFN) from DCs
   - Poor adjuvant for adaptive immune responses

2. Conjugation of a TLR7/8 agonist to HIV Gag protein induces multi-functional Th1 CD4+ T cells and CD8+ T cells in mice and NHP



--- Linker --- Gag protein

Conjugation of a TLR agonist to protein mimics infection by providing antigen and TLR stimulus to the same cell

Mechanisms by Which the Protein-TLR7/8 Conjugate Induces Multi-Functional Th1 and CD8 Responses

1. How does conjugation influence uptake of antigen by DCs?

2. Immunogenicity: How does the conjugate vaccine influence Th1 and CD8 priming *in vivo?*

    - Role of co-delivery of antigen and TLR 7/8 agonist

    - Role of cytokines (IL-12, Type I IFN) and TLR 7 signaling

1. Which DC subsets present and cross-present antigen?

Experimental Protocol

AF488-OVA ----**TLR7/8 agonist (conjugate vaccine)**

<u>Footpad Immunization (SQ)</u>

10 μg of OVA Protein +/- TLR 7/8 agonist

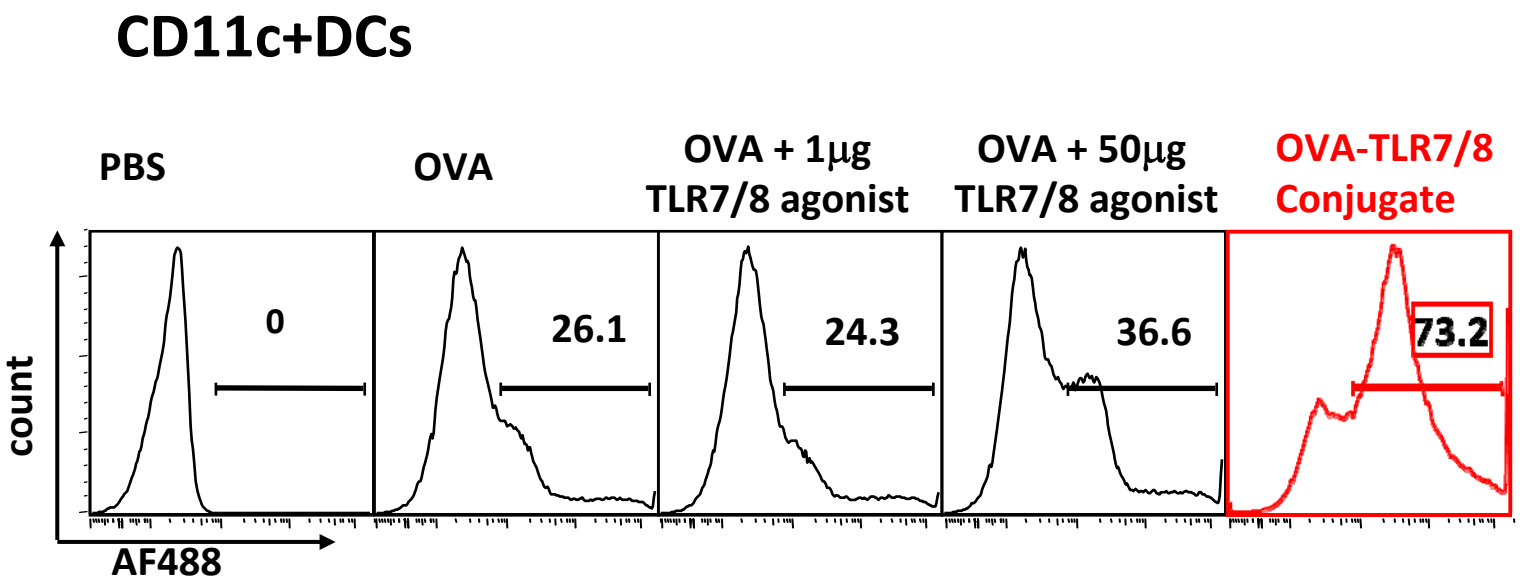
or

10 μg of OVA-TLR7/8 Conjugate

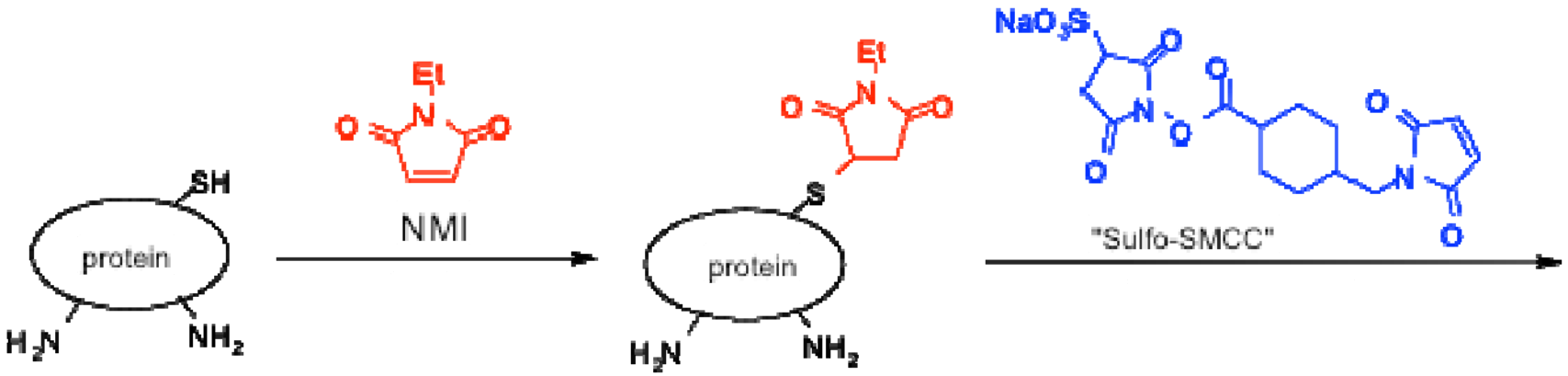
Draining Lymph Node (DC analysis)

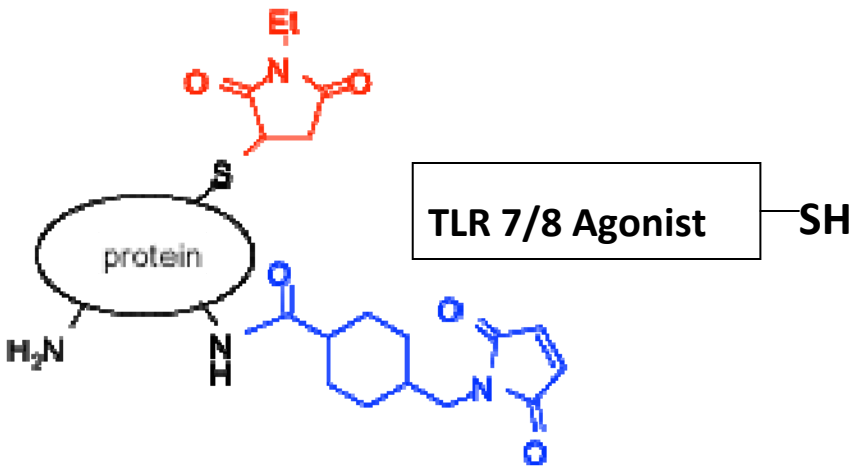
Spleen (T cell analysis)

Uptake of Conjugate Vaccine is More Efficient than Protein + Free TLR7/8 Agonist

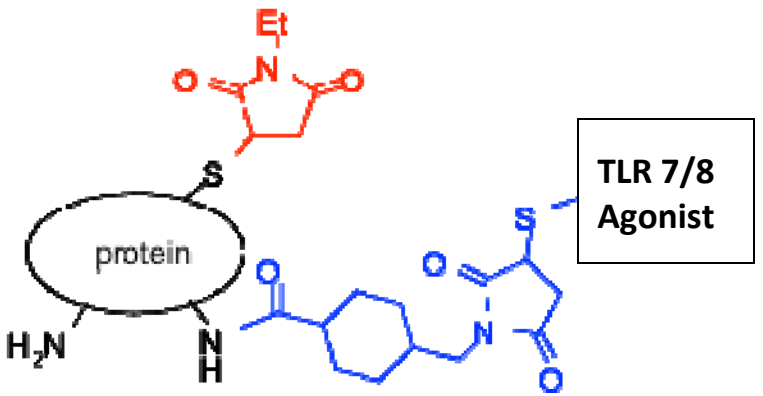


Method of Conjugating Protein to the TLR 7/8 Agonist





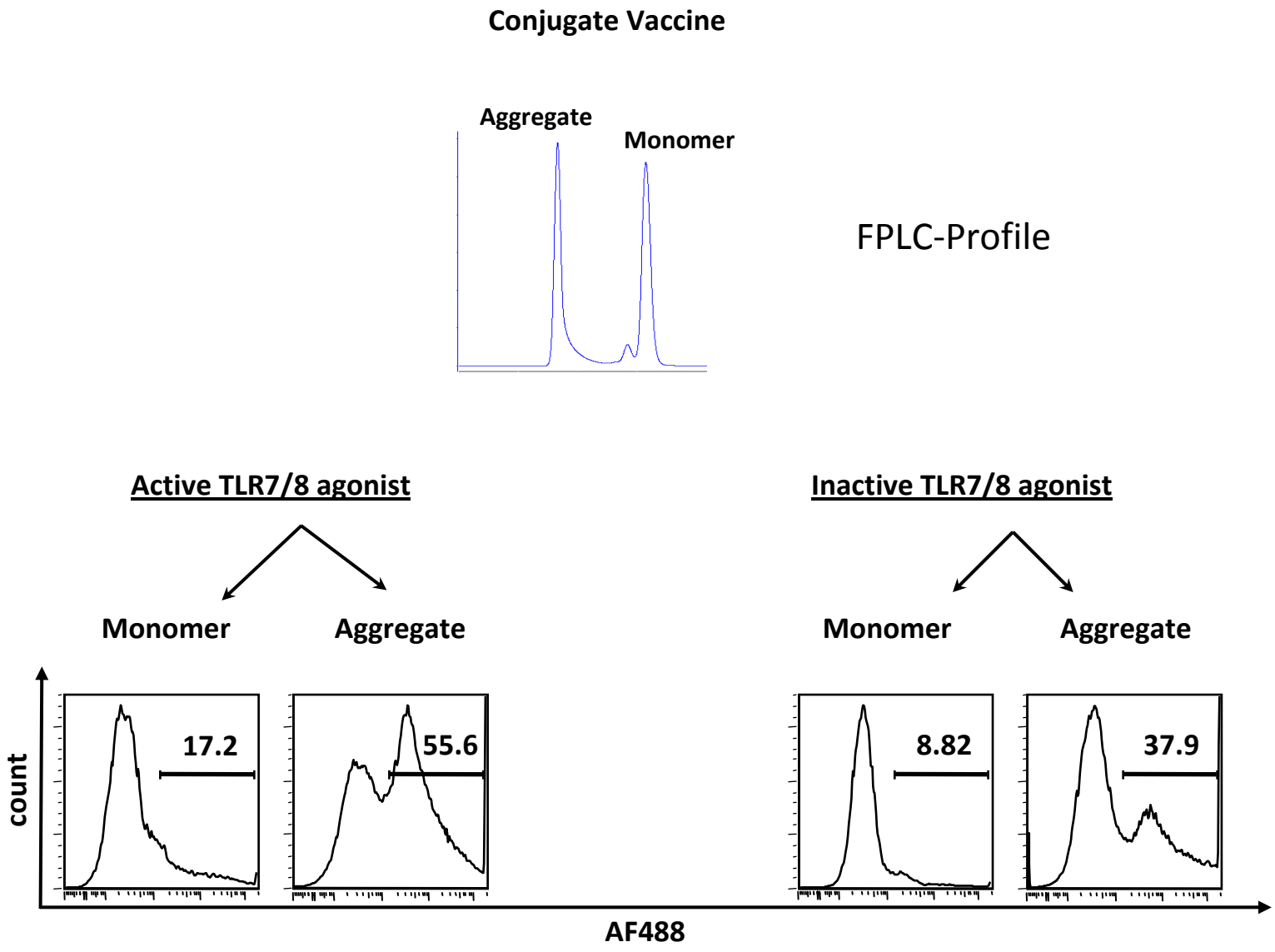
TLR 7/8 Agonist — SH



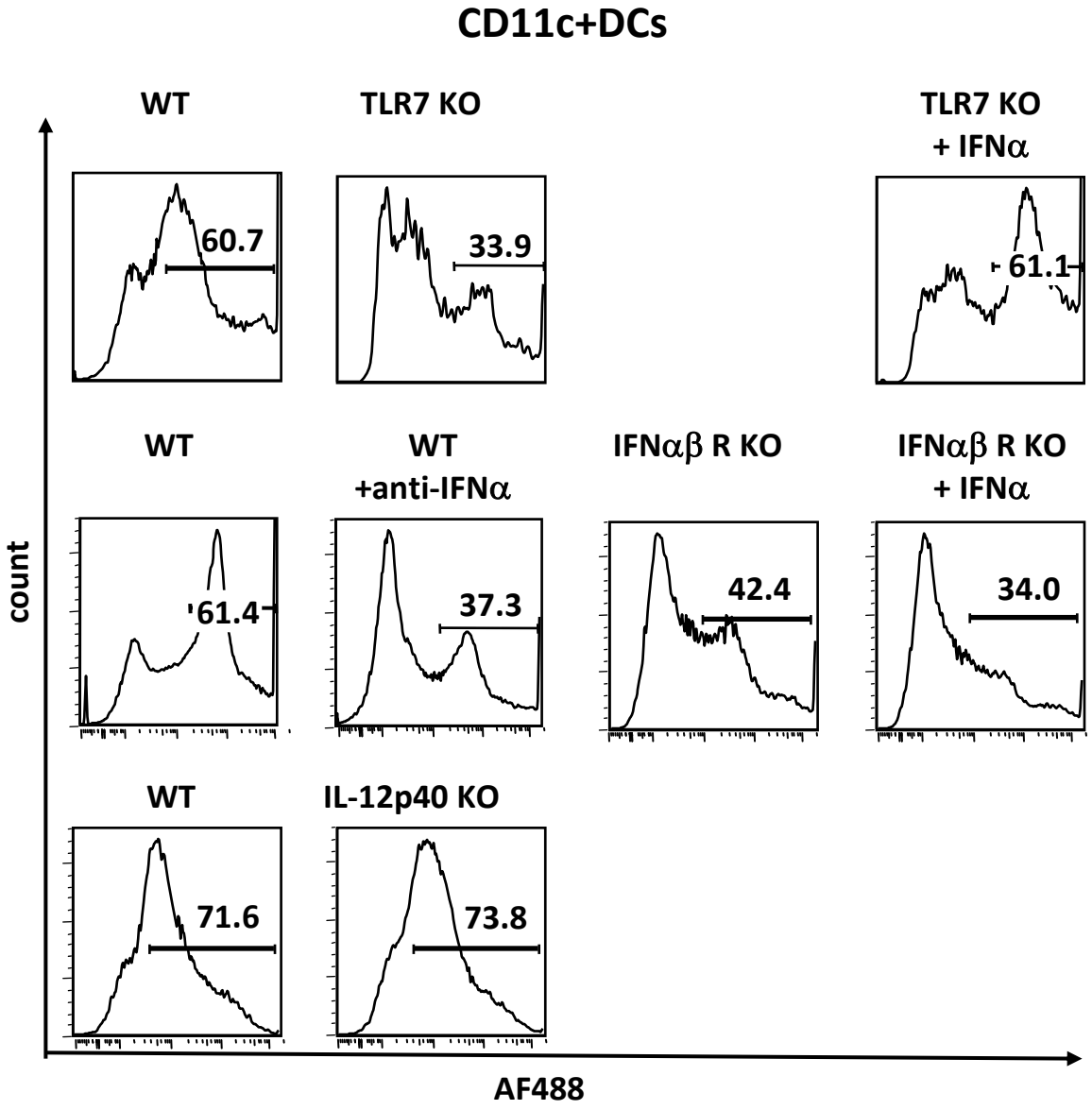
TLR 7/8 Agonist

Product = protein – sSMCC – TLR 7/8 Agonist

Optimal Uptake of the OVA-TLR 7/8 Conjugate Requires Aggregation *and* an Active TLR 7/8 Agonist



Optimal Uptake of the OVA-TLR 7/8 Conjugate Requires TLR 7 Signaling *and* Type I IFN *in vivo*



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1. How does conjugation influence uptake of antigen by DCs?

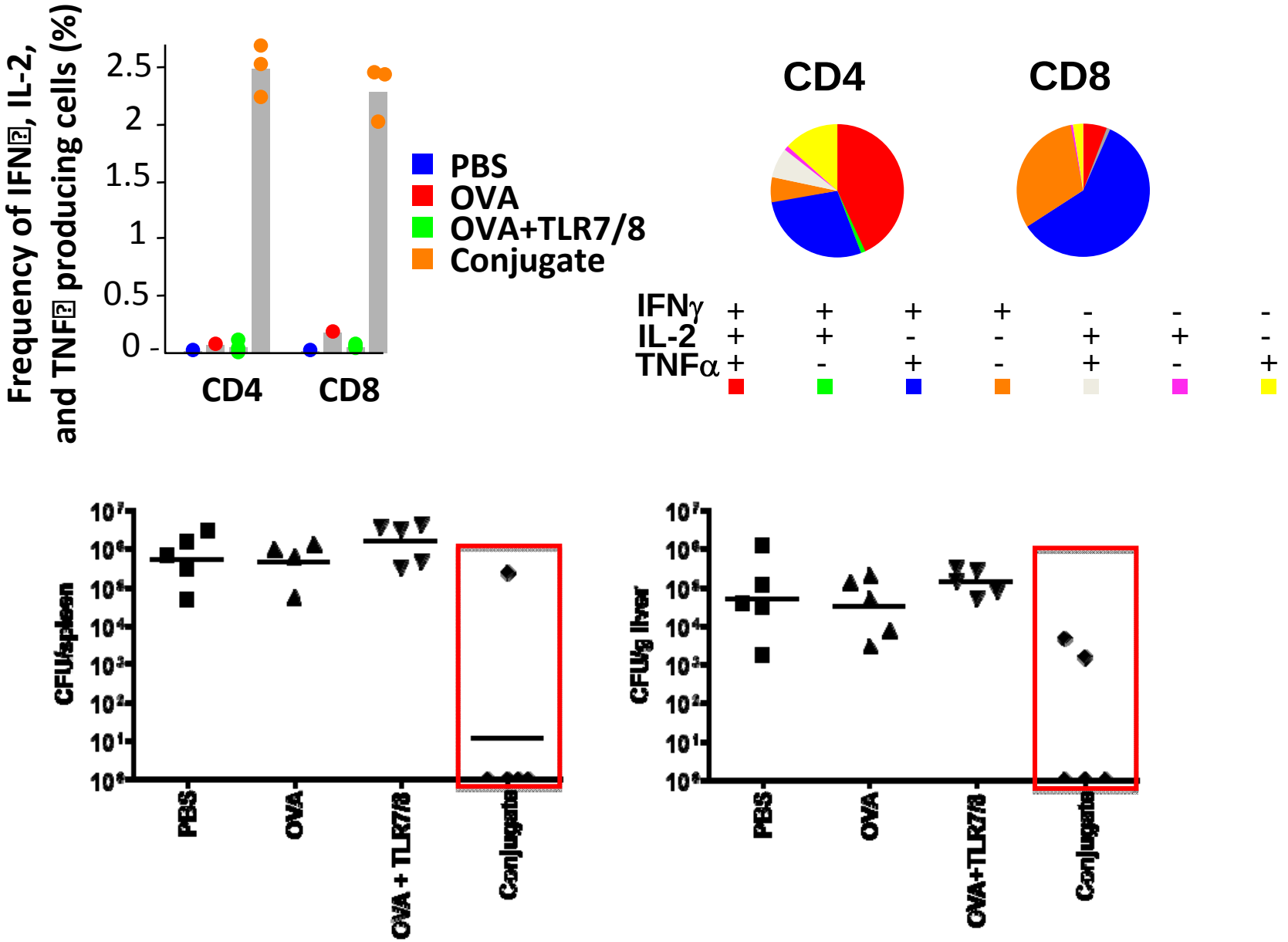
2. **Immunogenicity: How does the conjugate vaccine influence Th1 and CD8 priming *in vivo?***

    - **Role of co-delivery of antigen and TLR 7/8 agonist**

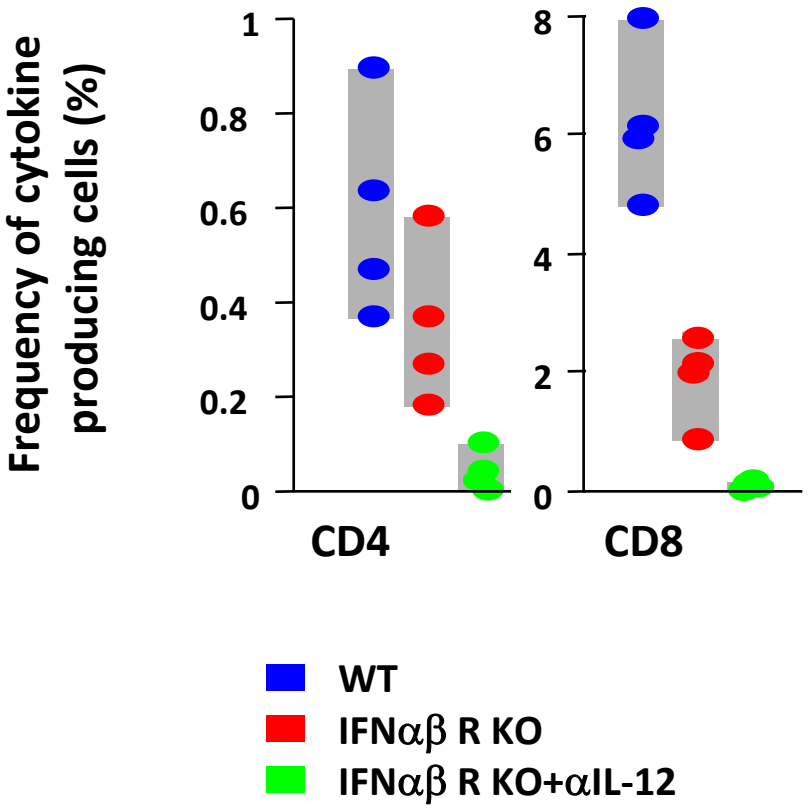
    - **Role of cytokines (IL-12, Type I IFN) and TLR 7 signaling**

1. Which DC subsets present and cross-present antigen?

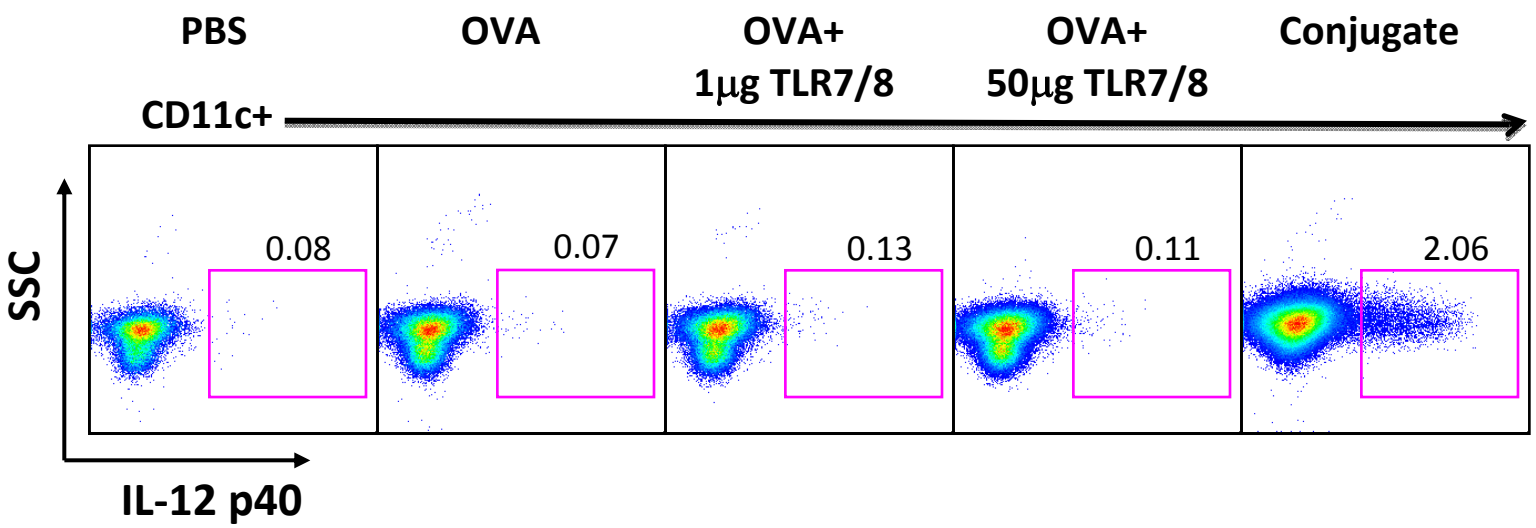
Conjugate Immunization Induces Protection Against *Listeria monocytogenes* Infection



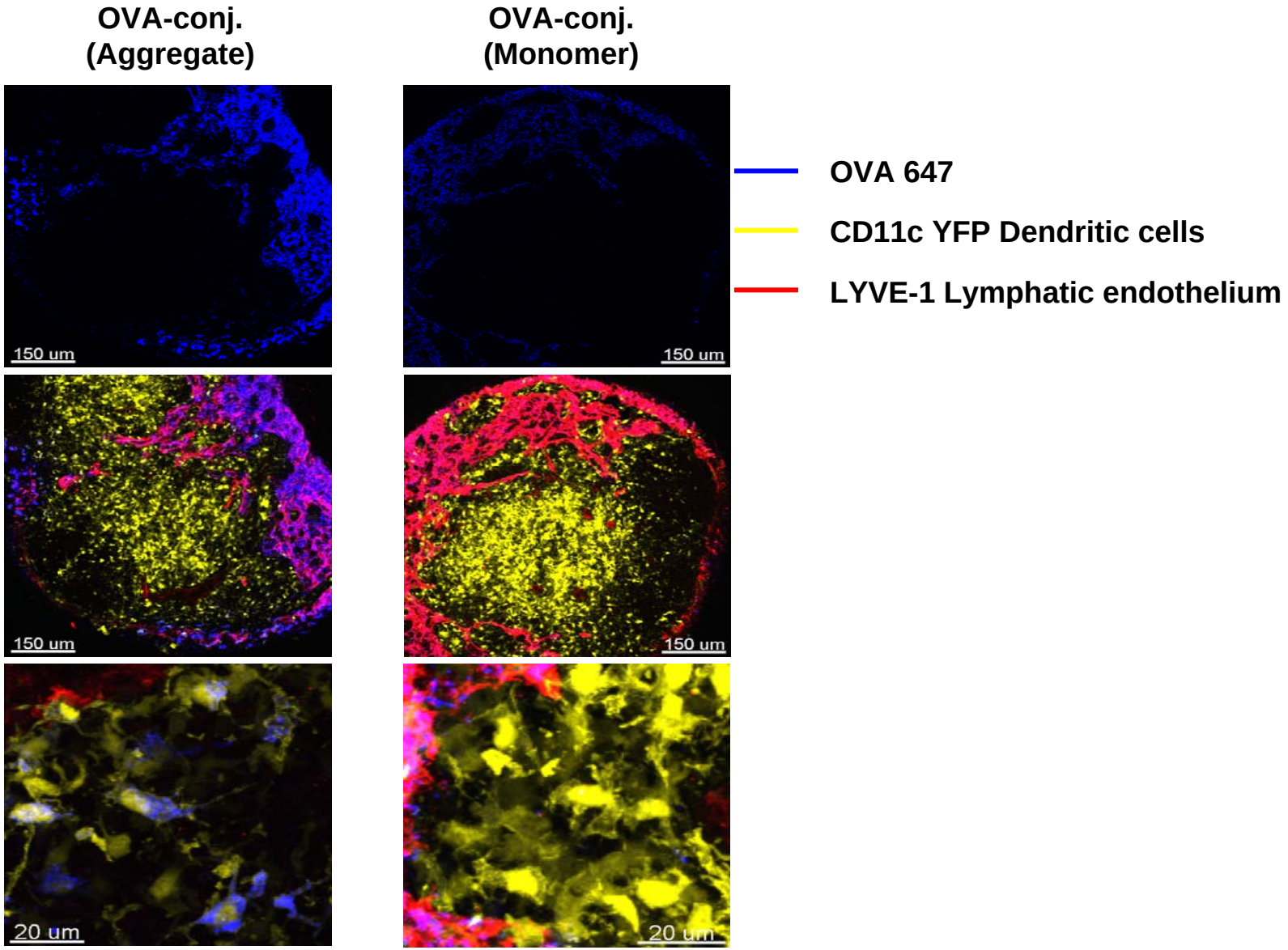
IL-12 *and* Type I IFN are Required for T Cell Immunity



Conjugate Vaccine Induces IL-12p40 by CD11c+CD8- DCs



Aggregated Conjugate Vaccine Accumulates in DLN



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    - Role of co-delivery of antigen and TLR 7/8 agonist

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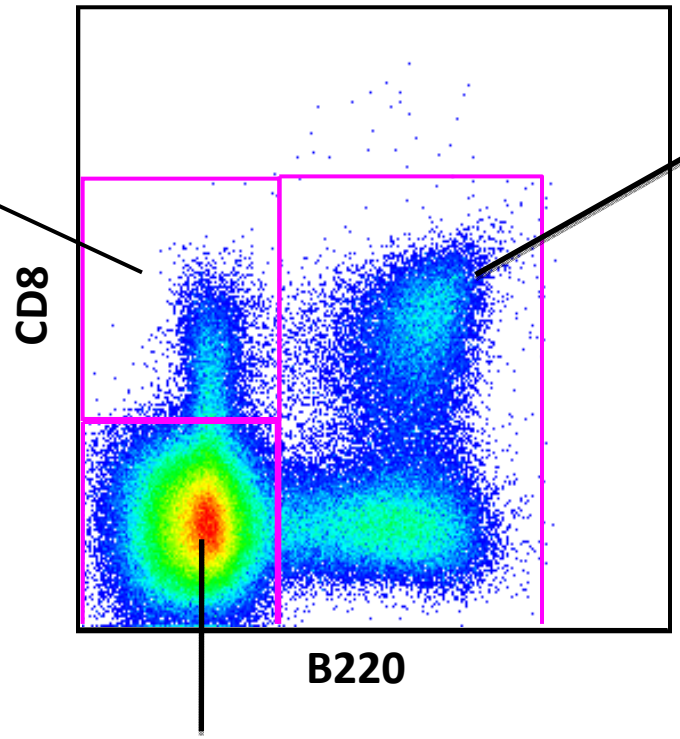
1. **Which DC subsets present and cross-present antigen?**

Major DC Subsets in Mice



CD8+ DC

Cross-priming

Plasmacytoid DC

Express TLR-7

IFN-α

? Ag presentation

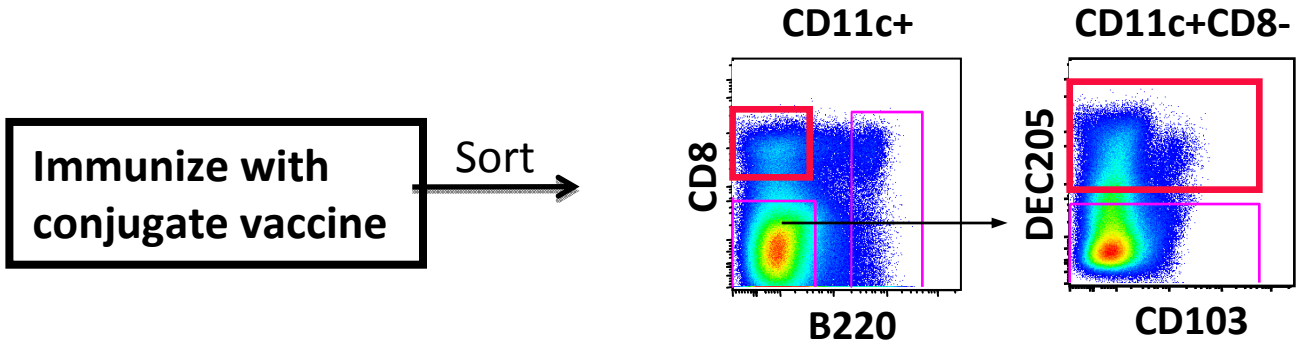
CD8- DC

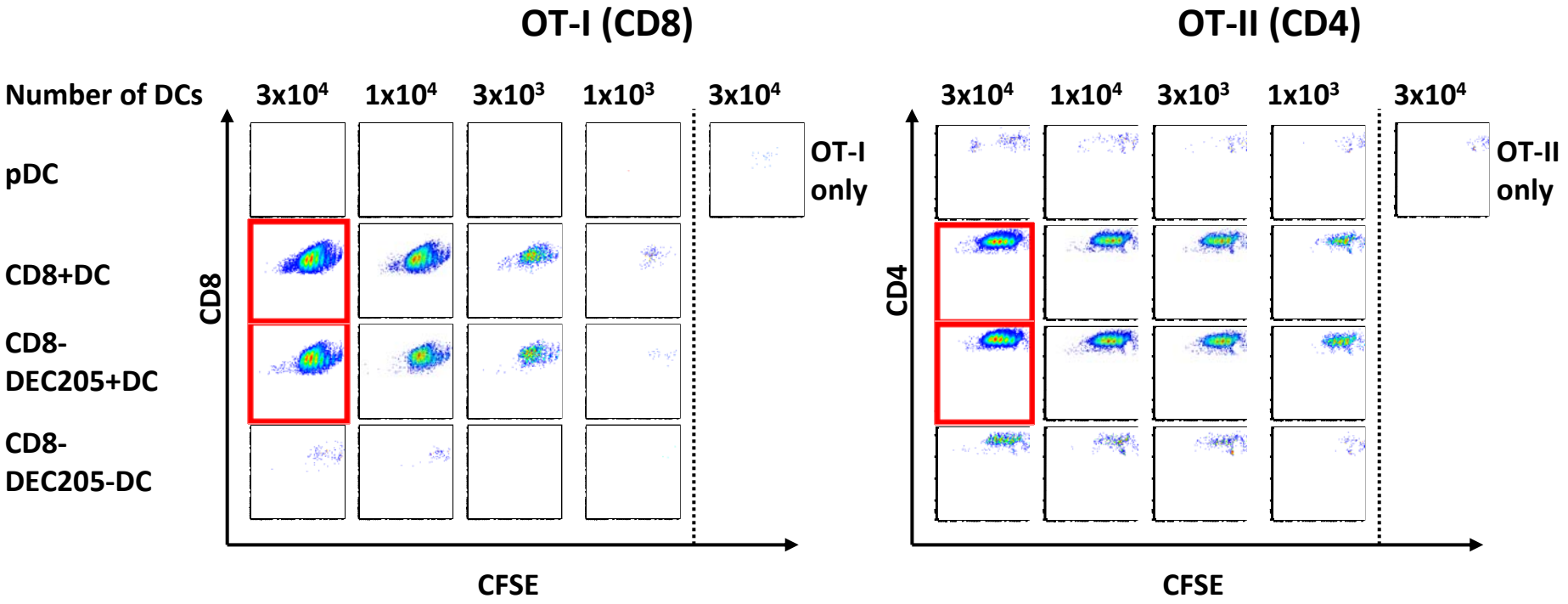
Express TLR-7

IL-12

MHC class II Ag presentation

CD8^{+} and CD8^{-}DCs Induce CD4 and CD8 T Cell Proliferation

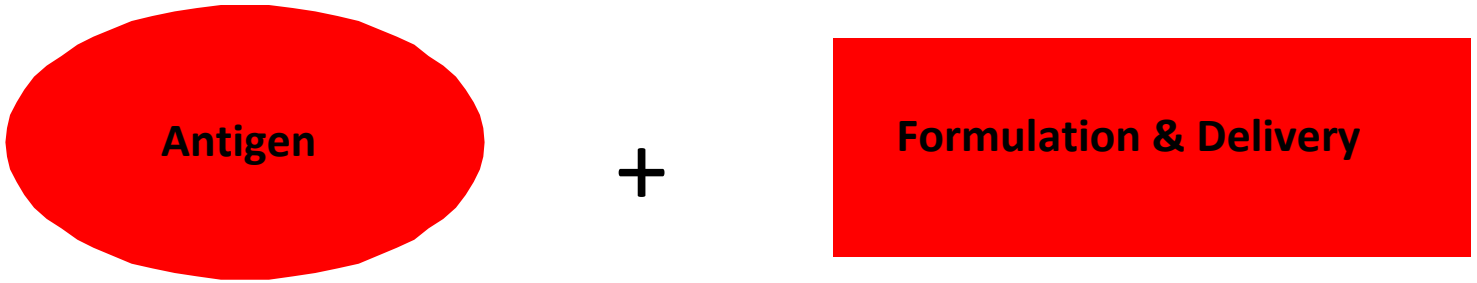




Summary

1. **Formulation**
   - Aggregation of protein improves uptake by DCs and is required for maximal T cell immunity with a TLR 7/8 agonist
   - TLR7 activation through Type I IFN increases the number and migration of DCs into DLN and enhances uptake of antigen
2. **Multiple DC subsets are required for optimal T cell immunity**
   - CD8- and CD8+ DCs mediate Th1 immunity
   - CD8+ DCs *and* CD8-dermal DCs induce CD8 T cells
   - pDCs have little antigen presenting capacity but provide Type I IFN
3. **Co-delivery of antigen and adjuvant to the same DC is useful approach for optimizing T cell immunity with TLR 7/8 ligands**

Optimizing T Cell Responses With Protein Vaccines Requires Formulation and Adjuvants



Specificity

Adjuvant Formulation

- **Vehicle**-Oil/water (MF 59), Alum, Liposomes, ISCOMS
- **Conjugation-**Physically couple protein to the adjuvant (TLR ligand)
- **Targeting**-Protein linked to antibody specific to dendritic cells

Optimizing T Cell Responses With Protein Vaccines Requires Improved Delivery

Hypothesis: To improve vaccine efficacy, vaccines should be targeted to appropriately mature DCs

1. How does targeting HIV Gag to DCs influence T cell immunity compared to untargeted protein?

1. Is Poly ICLC a suitable adjuvant to induce T and B cell responses in non-human primates?

Potential Receptors to Enhance Delivery of Antigens to Dendritic Cells

Langerin (CD207)
Dectin-1,2
DCIR, DCAR
DC-SIGN (CD209)
Clec-9/DNG R1
MMR (CD206)
DEC-205 (CD205)

↓

Endocytic receptor: C-type lectin that binds carbohydrates and mediates endocytosis.

DEC-205 (CD205) is expressed by cDCs, a major DC subset in the T cell areas of lymphoid tissues.

➡ **αDEC mAB that delivers Ag to cDC**

Targeting Vaccines to Dendritic Cells by Engineering Antigen into α-Human/ Rhesus DEC-205 Monoclonal Ab

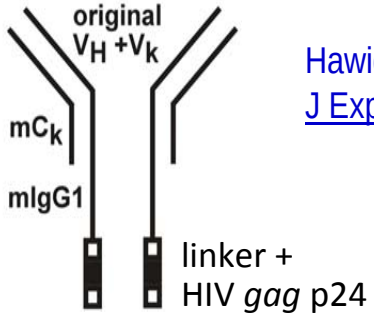
Genetic engineering of *gag* p24 protein into C-Terminus of a-human DEC205 heavy chain

↓

Co-transfect fusion heavy and light chains into 293 T cells

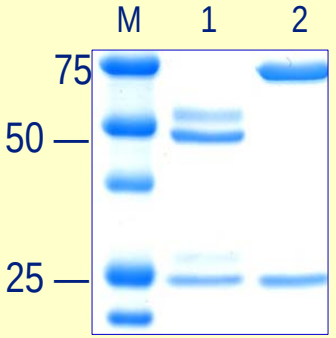
↓

protein G antibody purification



Hawiger *et al*,
J Exp Med (2001)

Antibodies Analyzed by SDS PAGE Under Reducing Conditions



Coomassie

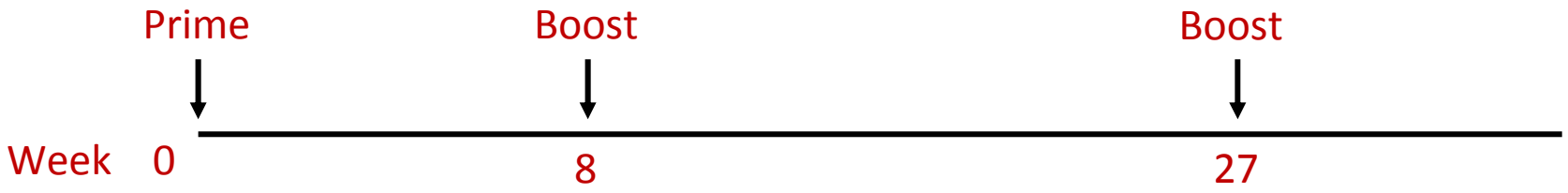
1 — α-DEC (Empty)
2 — α-DEC-p24

Poly I:C is a Potent Adjuvant for Inducing T and B Cell Responses

- Synthetic double-stranded RNA
- Agonist for TLR3 *and* MDA-5 innate signaling pathways
- **Strong inducer of Th1 cellular immunity**
- **Induces CD8 T cells through cross-presentation**
- Enhances humoral immunity by enhancing DC activation
- Poly ICLC is currently in multiple phase I trials for cancer

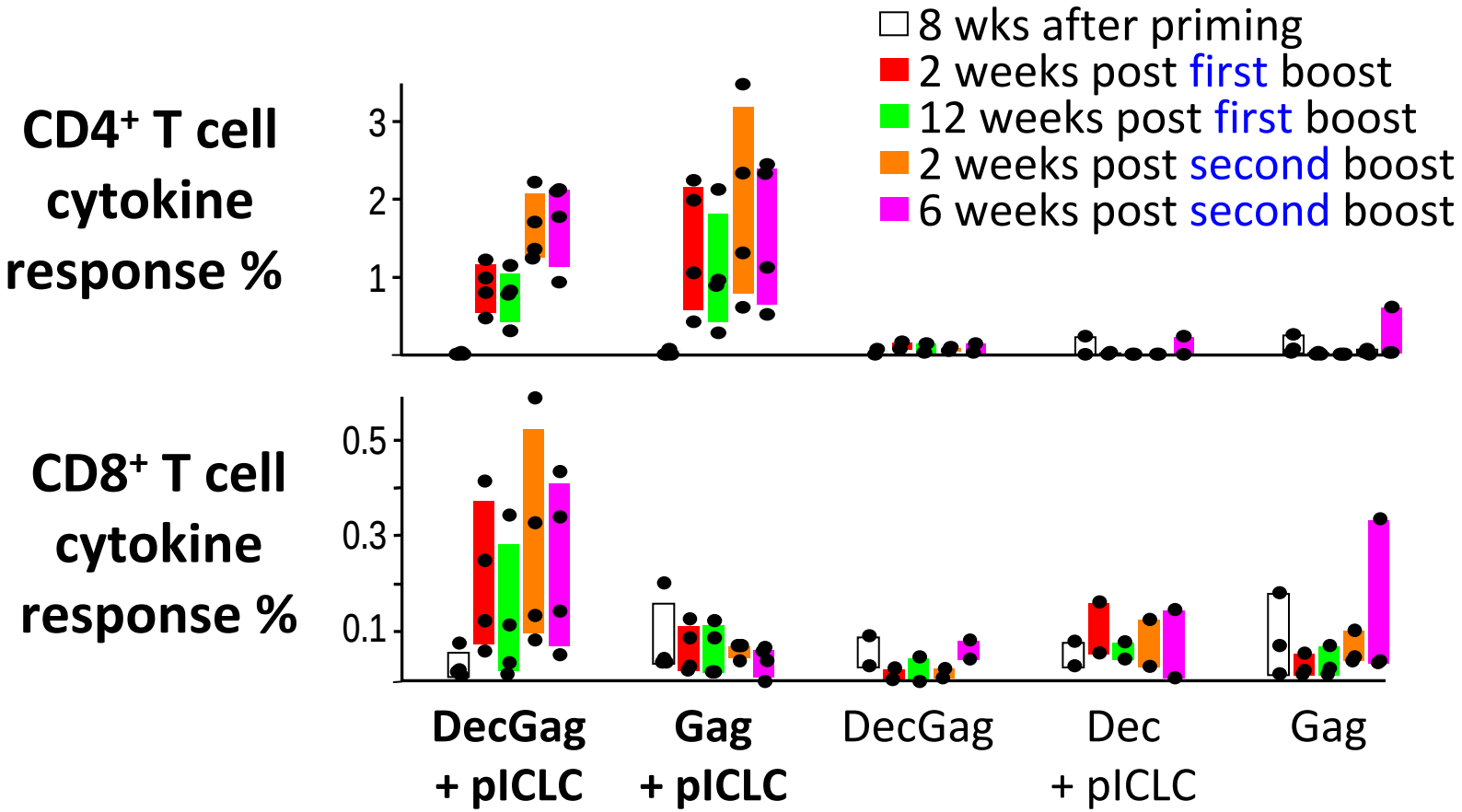
NHP Immunogenicity Study:
DEC Targeted vs. Non-Targeted HIV Gag p24 + poly ICLC

Group	Vaccines	N
1	α-Dec Gag p24 + Poly ICLC	4
2	Gag p24 + Poly ICLC	4
3	Gag p24 Protein alone	3
4	α-Dec Gag p24 alone	2
5	Empty α-Dec + Poly ICLC	2

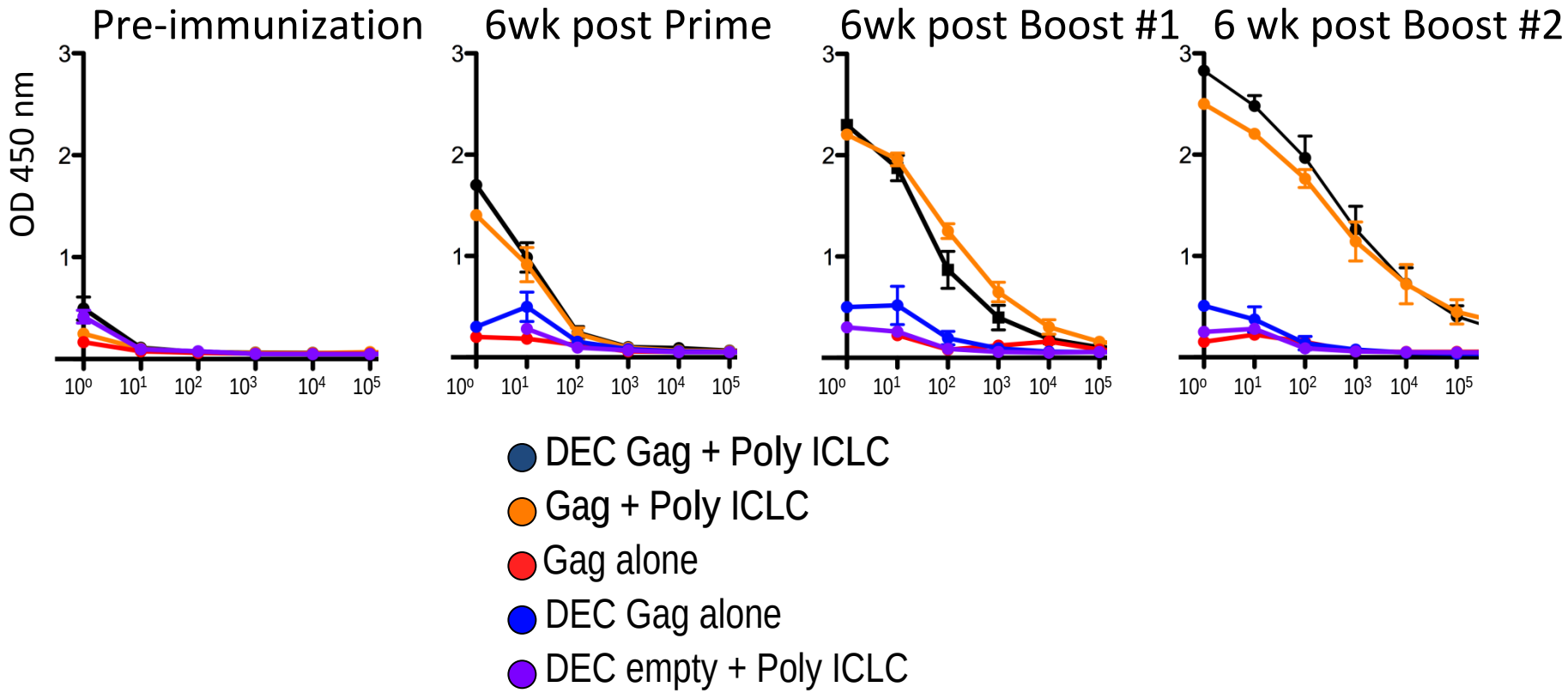


200 μg DEC-Gag and 60 μg Gag Protein are given SC +/- 1 mg/ml Poly ICLC

Magnitude: DEC Gag Plus Poly ICLC Is More Effective than Gag Plus Poly ICLC in Generating CD8+ T Cell Immunity



Anti-Gag Antibody Responses Are Strong to Both DEC Gag and Gag Protein Vaccines but Require Adjuvant



Surface Plasmon Resonance binding analyses revealed higher avidity responses in Gag + Poly ICLC immunized animals vs. DEC Gag plus Poly ICLC immunized animals

Summary

1. Poly ICLC is an effective adjuvant for inducing humoral and cellular immunity with non-targeted and DC targeted protein vaccines

2. The magnitude, breadth and quality of $CD4^{+/}$ Th1 responses were comparable with both targeted and non-targeted protein vaccines

3. Dendritic cell targeted vaccination better induced $CD8^{+}$ T cells

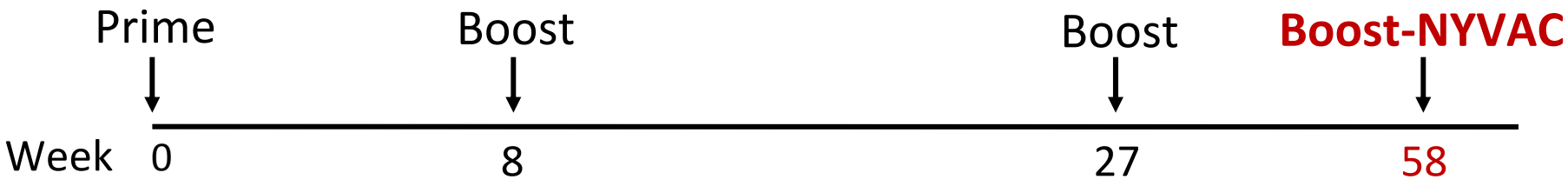
4. Both protein vaccines induced high titers of Gag-specific antibodies, but Gag protein + Poly ICLC induced higher avidity antibodies

Question:

Can HIV Gag protein vaccines prime for a single immunization with a viral vector boost?

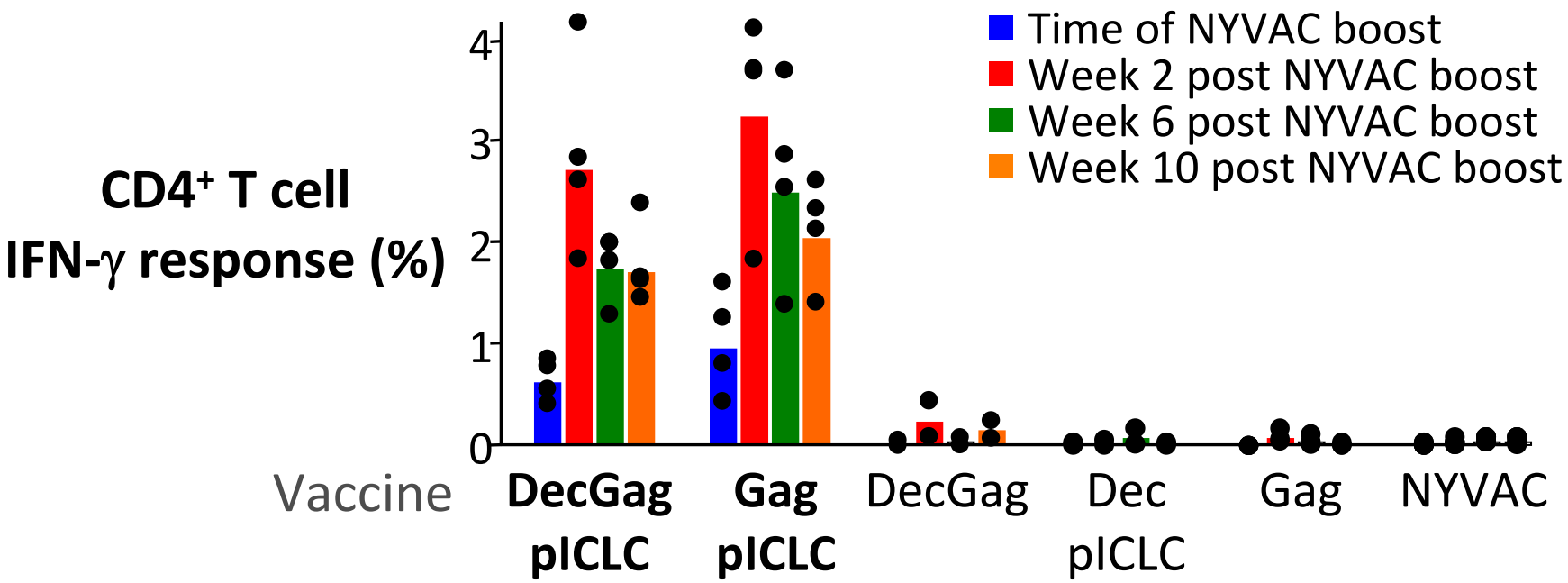
NHP Immunogenicity Study: NYVAC-Gag Boost of DEC Targeted vs. Non-Targeted HIV Gag p24 + Poly ICLC

Group	Prime	Boost	N
1	α-Dec Gag p24 + Poly ICLC	NYVAC	4
2	Gag p24 + Poly ICLC	NYVAC	4
3	Gag p24 Protein alone	NYVAC	3
4	α-Dec Gag p24 alone	NYVAC	2
5	Empty α-Dec + Poly ICLC	NYVAC	2
6	Poly ICLC	NYVAC	6



1 X 10^8 PFU NYVAC was given once i.m per animal

A Single Dose of NYVAC-HIV Gag Boosts CD4+ T Cells in NHP Primed to Targeted or Non-targeted Gag Protein + Poly ICLC



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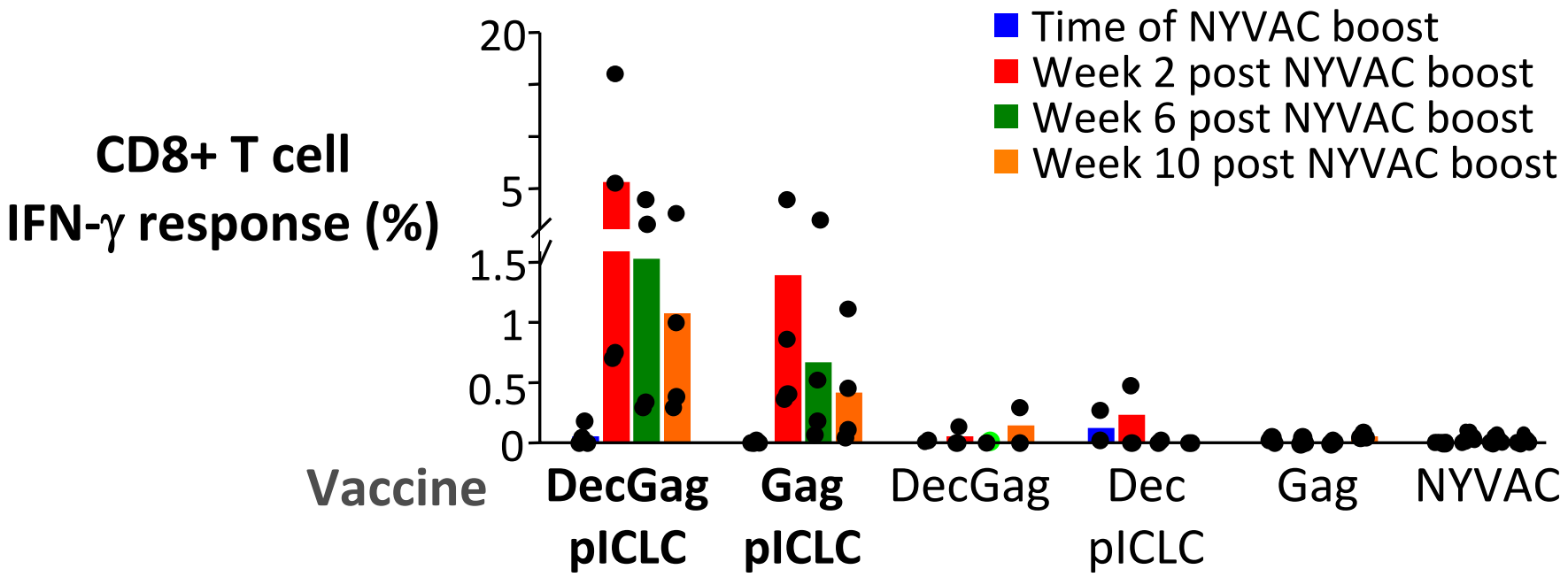

Summary

1. **Protein vaccines can dramatically improve the efficacy of a recombinant NYVAC viral vector for T cell immunity**

   -Cross primed CD8+ T cells are potently boosted

1. **NYVAC should be used as a boost for optimizing T cell immunity with protein and other vaccines**

Formulation and Delivery Influence Adaptive Immunity

Targeting

Conjugation

Non-Targeted

Immune Correlates of Protection

Disease	Immune Correlate	Best Vaccine
M. tuberculosis	Th1,?CD8	BCG
L. major	Th1,?CD8	Leishmania
Malaria	Ab, CD8, Th1	Irradiated sporozoites
HIV	Ab, CD8,CD4	CMV in NHP

All of these are live vaccines

Qualities of Ralph Steinman

- Steadfast
- Rigorous
- Tireless
- Optimistic
- Supportive
- Was very critical of funding mechanisms

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