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
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

<table>
<thead>
<tr>
<th>TLR Expression</th>
<th>TLR Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR 4</td>
<td>-</td>
</tr>
<tr>
<td>TLR 3</td>
<td>+</td>
</tr>
<tr>
<td>TLR 7</td>
<td>-</td>
</tr>
<tr>
<td>TLR 8</td>
<td>+</td>
</tr>
<tr>
<td>TLR 9</td>
<td>-</td>
</tr>
</tbody>
</table>

*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

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 \textit{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)

Footpad Immunization (SQ)
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

CD11c+DCs

PBS  OVA  OVA + 1µg TLR7/8 agonist  OVA + 50µg TLR7/8 agonist  OVA-TLR7/8 Conjugate

AF488  count

0  26.1  24.3  36.6  73.2
Method of Conjugating Protein to the TLR 7/8 Agonist

Optimal Uptake of the OVA-TLR 7/8 Conjugate Requires \textbf{Aggregation \textit{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

<table>
<thead>
<tr>
<th>CD11c+DCs</th>
<th>WT</th>
<th>TLR7 KO</th>
<th>TLR7 KO + IFNα</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>AF488 WT 60.7</td>
<td>AF488 TLR7 KO 33.9</td>
<td>AF488 TLR7 KO + IFNα 61.1</td>
</tr>
<tr>
<td>WT + anti-IFNα</td>
<td>AF488 WT 61.4</td>
<td>AF488 WT 37.3</td>
<td>AF488 IFNαβ R KO 42.4</td>
</tr>
<tr>
<td>IL-12p40 KO</td>
<td>AF488 WT 71.6</td>
<td>AF488 IL-12p40 KO 73.8</td>
<td>AF488 IFNαβ R KO + IFNα 34.0</td>
</tr>
</tbody>
</table>
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 \textit{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

- **Frequency of IFN-γ, IL-2, and TNF-α producing cells (%):**
- **Graphs:**
  - CD4 and CD8 categories with different immunization conditions.
  - Comparison of CFU in spleen and liver across PBS, OVA, OVA+TLR7/8, and Conjugate groups.

**Figure Details:**
- **CD4** and **CD8** categories.
- **IFN-γ**, **IL-2**, and **TNF-α** production variations across groups.
- **Immunization Induces Protection Against** *Listeria monocytogenes* Infection.
IL-12 and Type I IFN are Required for T Cell Immunity

Frequency of cytokine producing cells (%)

CD4

CD8

WT
IFNαβ R KO
IFNαβ R KO+αIL-12
Conjugate Vaccine Induces IL-12p40 by CD11c+CD8- DCs
Aggregated Conjugate Vaccine Accumulates in DLN

OVA-conj. (Aggregate)  OVA-conj. (Monomer)

- OVA 647
- CD11c YFP Dendritic cells
- LYVE-1 Lymphatic endothelium
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 \textit{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?
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**

Immune system cells are visualized in a flow cytometry diagram. The diagram shows the sorting of DCs based on CD11c and CD8 expression, with different groups of DCs (pDC, CD8⁺DC, CD8⁻DEC205⁺DC, CD8⁻DEC205⁻DC) and their interaction with T cells (OT-I and OT-II) labeled with CFSE. The number of DCs varies from 3x10⁴ to 1x10³ across different conditions.
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

Antigen + Formulation & Delivery

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
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 $\alpha$-Human/ Rhesus DEC-205 Monoclonal Ab

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

\[ \downarrow \]

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

\[ \downarrow \]

protein G antibody purification

Antibodies Analyzed by SDS PAGE Under Reducing Conditions

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Vaccines</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$\alpha$-Dec Gag p24 + Poly ICLC</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Gag p24 + Poly ICLC</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Gag p24 Protein alone</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>$\alpha$-Dec Gag p24 alone</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Empty $\alpha$-Dec + Poly ICLC</td>
<td>2</td>
</tr>
</tbody>
</table>

Prime:
Week 0

Boost:
Week 8

Boost:
Week 27

200 $\mu$g DEC-Gag and 60 $\mu$g 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

CD4⁺ T cell cytokine response %

CD8⁺ T cell cytokine response %

- DecGag + pICLC
- Gag + pICLC
- DecGag
- Dec + pICLC
- Gag

Legend:
- 8 wks after priming
- 2 weeks post first boost
- 12 weeks post first boost
- 2 weeks post second boost
- 6 weeks post second boost
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

<table>
<thead>
<tr>
<th>Group</th>
<th>Prime</th>
<th>Boost</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>α-Dec Gag p24 + Poly ICLC</td>
<td>NYVAC</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Gag p24 + Poly ICLC</td>
<td>NYVAC</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Gag p24 Protein alone</td>
<td>NYVAC</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>α-Dec Gag p24 alone</td>
<td>NYVAC</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Empty α-Dec + Poly ICLC</td>
<td>NYVAC</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>Poly ICLC</td>
<td>NYVAC</td>
<td>6</td>
</tr>
</tbody>
</table>

**Prime**

<table>
<thead>
<tr>
<th>Week</th>
<th>Prime</th>
<th>Boost</th>
<th>Boost</th>
<th>Boost-NYVAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>8</td>
<td>27</td>
<td>58</td>
</tr>
</tbody>
</table>

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 IC LC

CD4+ T cell IFN-γ response (%)

Vaccine
DecGag pICLC Gag pICLC DecGag Dec pICLC Gag NYVAC

Time of NYVAC boost
Week 2 post NYVAC boost
Week 6 post NYVAC boost
Week 10 post NYVAC boost
A Single Dose of NYVAC-HIV Gag Boosts $\text{CD8}^+\ T$ Cells in NHP Primed to Targeted or Non-Targeted Gag Protein + Poly ICLC
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

<table>
<thead>
<tr>
<th>Targeting</th>
<th>Conjugation</th>
<th>Non-Targeted</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.jpg" alt="Targeting Image" /></td>
<td><img src="image2.jpg" alt="Conjugation Image" /></td>
<td><img src="image3.jpg" alt="Non-Targeted Image" /></td>
</tr>
</tbody>
</table>
# Immune Correlates of Protection

<table>
<thead>
<tr>
<th>Disease</th>
<th>Immune Correlate</th>
<th>Best Vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. tuberculosis</em></td>
<td>Th1, CD8</td>
<td>BCG</td>
</tr>
<tr>
<td><em>L. major</em></td>
<td>Th1, CD8</td>
<td>Leishmania</td>
</tr>
<tr>
<td><em>Malaria</em></td>
<td>Ab, CD8, Th1</td>
<td>Irradiated sporozoites</td>
</tr>
<tr>
<td><em>HIV</em></td>
<td>Ab, CD8, CD4</td>
<td>CMV in NHP</td>
</tr>
</tbody>
</table>

All of these are live vaccines
Qualities of Ralph Steinman

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

<table>
<thead>
<tr>
<th>Vaccine Research Center, NIAID</th>
<th>Dermatology Branch, NCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kathrin Kastenmueller</td>
<td>Mark Udey</td>
</tr>
<tr>
<td>Kylie Quinn</td>
<td>Maria Becker</td>
</tr>
<tr>
<td>Ross Lindsay</td>
<td>Wolfgang Kastenmueller</td>
</tr>
<tr>
<td>Barbara Flynn</td>
<td>Laboratory of Immunology, NIAID</td>
</tr>
<tr>
<td>Kavita Tewari</td>
<td>Ron Germain</td>
</tr>
<tr>
<td>Tricia Darrah</td>
<td>Erasmus University Medical Center</td>
</tr>
<tr>
<td>Sonia Hegde</td>
<td>Bjorn Clausen</td>
</tr>
<tr>
<td>Smita Chandran</td>
<td>University of Colorado</td>
</tr>
<tr>
<td>Andreia Costes</td>
<td>Ross Kedl</td>
</tr>
<tr>
<td>Lauren Trager</td>
<td>Jason Oh</td>
</tr>
<tr>
<td>Ulli Wille-Reece (PATH-MVI)</td>
<td>University of Minnesota</td>
</tr>
<tr>
<td></td>
<td>Dan Kaplan</td>
</tr>
<tr>
<td></td>
<td>Botond Igyarto</td>
</tr>
<tr>
<td>Rockefeller University</td>
<td>Centro Nacional de Biotecnologia, Madrid, Spain</td>
</tr>
<tr>
<td>Ralph Steinman (late)</td>
<td>Mariano Esteban</td>
</tr>
<tr>
<td>Michel Nussensweig</td>
<td></td>
</tr>
<tr>
<td>Christine Trumpfeller</td>
<td></td>
</tr>
<tr>
<td>Tibor Kellor (Celldex Therapeutics)</td>
<td></td>
</tr>
</tbody>
</table>