Strategies and Opportunities for Cancer Therapy with Vaccines Inducing T cells or Antibodies

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NCAB Meeting
Bethesda, Maryland
June 17, 2008
Rationale for Engineered Vaccines

- Most successful vaccines (except toxoids) have been against viruses causing acute, self-limited infections, for which the most widely used strategy is to mimic the natural infection with an attenuated, inactivated, or subunit vaccine.

- However, for cancer or viruses causing chronic infection, such as HIV or hepatitis C virus, the natural disease does not induce sufficient immunity to eradicate the infection.

- A vaccine must elicit better immunity than the disease itself.
CD8⁺ Cytotoxic T cells can detect endogenous antigenic proteins even if not expressed intact on the cell surface.
# Types of Tumor Antigens

**Examples**

- **Overexpressed antigens**
  - Her-2/neu, CEA, TARP

- **Altered antigens**
  - Shared by many tumors: p53, Ras, fusion proteins, MUC1
  - Unique to a single tumor: Point mutations in various genes

- **Tissue-specific antigens**
  - tyrosinase, MART1, gp100

- **Novel antigens (in adult)**
  - Fetal antigens: CEA, oncofetal protein
  - Viral antigens: HPV E6 or E7, EBV antigens
  - Clonal antigens: Idiotype
Desirable Characteristics for Tumor Antigens

1. Tumor-selective

2. Essential to tumor cell survival

3. For T-cell antigens
   - Processed
   - Bind MHC
   - Immunogenic

4. For B-Cell antigens
   - Cell Surface Expression
   - Accessibility of Epitopes
   - Immunogenicity
     - In vitro
     - In vivo
     Pre-existing Antibody response
Potential Mechanisms of Antibody Action against Tumors

• Antibody-dependent cellular cytotoxicity (ADCC): NK or other cells with Fc receptors bind antibodies and use them to target cells for killing.

• Complement-mediated lysis

• Inhibition of function of a molecule required for oncogenicity: e.g. HER-2/neu, CD25

• Success of antibodies to HER-2/neu (Herceptin) and to CD25 (Zenapax) suggests functional targets may be the most effective.
Adeno-neuECTM (Her-2) treatment causes regression of established s.c. TUBO mammary carcinomas

Park et al., Cancer Research 2008
Adeno-neuECTM (Her-2) vaccine induces regression of established lung tumors from IV injection of TUBO breast cancer cells

Park et al., Cancer Research 2008
Ad-neuECTM serum downmodulates ErbB2 (Her-2) and inhibits its phosphorylation

Park et al., *Cancer Research* 2008
Advantages of Vaccine over Trastuzumab (Herceptin)

• Antibody induced by vaccine is not dependent on FcRs, but directly inhibits the function of the oncogene product and inhibits tumor growth without other cells. Herceptin requires FcRs.

• Polyclonal antibodies elicited may target multiple Her-2 epitopes and be less susceptible to escape mutations than a monoclonal antibody to a single epitope.

• Continuous antibody production avoids the need for repeated expensive monoclonal antibody administration (~$100K/yr).
CD8⁺ Cytotoxic T cells can detect endogenous antigenic proteins even if not expressed intact on the cell surface.
# Cancer Vaccine Problems & Strategies

<table>
<thead>
<tr>
<th>Problems</th>
<th>Strategies to solve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self antigens to which host is tolerant</td>
<td>Target subdominant epitopes strengthened by epitope enhancement:</td>
</tr>
<tr>
<td></td>
<td>Modify the amino acid sequence to improve MHC binding.</td>
</tr>
<tr>
<td>Downregulation of MHC or of processing machinery</td>
<td>Induce higher avidity T cells that can respond to low densities of peptide-MHC</td>
</tr>
<tr>
<td>Poor quality or quantity of immune response</td>
<td>Use cytokines to improve the quantity and quality and substitute for CD4⁺ help:</td>
</tr>
<tr>
<td>For therapeutic vaccines, inadequate CD4⁺ T help</td>
<td></td>
</tr>
<tr>
<td>Suppression of the immune response</td>
<td>Remove the brakes by blocking negative regulation.</td>
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</table>
PUSH-PULL Approach to Optimizing Vaccine-induced Immunity

Optimize
Antigen

Optimize
Cytokines
Costimulatory molecules
Toll-like receptor ligands

Block negative regulation (PULL)
Negative Regulators

Immune Response
Topics:

- Improve the antigen: epitope enhancement
- Use cytokines to improve the quality and quantity of immune response
- Improve CTL quality by increasing avidity with IL-15
- Improve CTL quality by using IL-15 to substitute for CD4^+ T cell help to induce long-lived memory CTL
- Remove the brakes by blocking negative regulation: A new NKT regulatory axis.
Peptide Fragments of Viral Proteins Bind Specifically in the Groove of Major Histocompatibility Molecules such as HLA-A, B, C

Sendai Virus Peptide Bound to H-2K$^b$


**Strategy:** Epitope Enhancement by Sequence Modification to Increase Peptide Affinity for the MHC Molecule
Major Histocompatibility Molecule (HLA)
Enhanced Vaccine Protects Against Higher Viral Challenge

Ahlers et al., *JCI* 108:1677, 2001
TARP: TCRγ Alternative Reading frame Protein

• Expressed in prostate and breast cancers, but not in other organs
• Using different open reading frame from normal TCRγ
• Possible role: Oncogenic transformation of the cells

Amino Acid Sequence of TARP

MQMFPPSPLFFFLQLLLKQSSRRLEHTFVFLRNFSLMLLLRGIGKKRRATRFWDPRRGTP (58 residues)

FLRNFSLMLML = HLA-A2-binding peptide
TARP 29-37
Human CTL raised against an epitope-enhanced TARP peptide can kill human tumor cells expressing TARP and HLA-A2.
Use of Cytokines in Adjuvant to Steer the Immune Response to Vaccines

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>CTL Proliferation</th>
<th>IL-2</th>
<th>IL-4</th>
<th>IFN-γ</th>
<th>Cytokine mRNA</th>
<th>Ab Isotype</th>
<th>Neutralizing Ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CSF</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td></td>
<td></td>
<td>IgG1, 2b</td>
<td>↑</td>
</tr>
<tr>
<td>IL-1β</td>
<td>↓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IgG1</td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>↑</td>
<td>↑</td>
<td></td>
<td></td>
<td></td>
<td>IgG2a</td>
<td>↑</td>
</tr>
<tr>
<td>IL-4</td>
<td>↓</td>
<td></td>
<td>↑</td>
<td></td>
<td></td>
<td>IgG1, 2b</td>
<td>↑</td>
</tr>
<tr>
<td>IL-7</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td></td>
<td></td>
<td>IgG1</td>
<td></td>
</tr>
<tr>
<td>IL-12</td>
<td>↑</td>
<td>↑/↑</td>
<td></td>
<td></td>
<td></td>
<td>IgG1, 2a, 2b</td>
<td>↑</td>
</tr>
<tr>
<td>TNFα</td>
<td>↓/↑</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>↑</td>
<td>↑</td>
<td></td>
<td></td>
<td></td>
<td>IgG2a, 2b</td>
<td></td>
</tr>
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</table>

Two peptide vaccine candidates: PCLUS 3-18MN, PCLUS 6.1-18-MN
Two mouse genetic backgrounds: BALB/c, B10 congenics

Cytokine Synergies:
IL-12 and GM-CSF synergized for CTL induction
Topics:

Use of IL-15 in the vaccine to induce high functional avidity CTL (recognizing low densities of peptide-MHC complexes on cells)
High, Intermediate, and Low **Functional Avidity** CTL Generated by Stimulation with Different Concentrations of Peptide Antigen

Hypothesis: high avidity CTL are more effective at killing tumor cells

High avidity CTL

Low avidity CTL

Cancer cell

Peptide-coated cell

Cancer cell

Peptide-coated cell

Only HIGH AVIDITY CTL kill tumor cells

- Control cells
- Peptide-coated cells
- Tumor cells

IL-2 & IL-15: DISTINCT SOURCE & FUNCTIONS

IL-15 (made by DC)
- Mast cell proliferation
- NK cell development
- Memory CD8 T cells
- Maintenance

IL-2 (made by T cells)
- Activated T cells
- Antigen-Induced Cell Death
Immunization with antigen + IL-15 induces higher functional avidity memory CD8\(^+\) CTL

Oh et al., PNAS 2004

2 months after immunization
Complementary Mechanisms for IL-15 in CTL Avidity Maturation

Oh et al. PNAS, 2004
Topics:

Use of IL-15 in the vaccine to induce high avidity CTL (recognizing low densities of peptide-MHC complexes on cells)

Improve CTL quality by using IL-15 to substitute for CD4+ T cell help to induce long-lived memory CTL
IL-15 expression by a vaccine vector induced longer-lived memory CD8⁺ CTL: IFN-gamma-producing cells

Explained by 1. Higher IL-15Rα expression
2. Greater homeostatic proliferation

Oh et al., *PNAS* 2003
CD4+ T-cell Help for CD8+ CTL Mediated Through Activation of Dendritic Cell

CD8+ Cytotoxic T cell

High avidity
Longevity

IL-15 with vaccine

CD4+ Helper T cell

CD4+ T-cell Help for CD8+ CTL Mediated Through Activation of Dendritic Cell

CD8+ Cytotoxic T cell

IL-15
IL-12

MHC Class I

CD40L

Dendritic Cell

TCR

IL-15

Oh et al.,

PNAS, 2008
IL-15 during immunization substitutes for CD4⁺ T cell help to induce long-lived memory CTL (One year after immunization)

Oh et al., PNAS, 2008
Conclusions for improving CTL quality

**IL-15 in a vaccine:**
- Induces longer-lived memory CD8 CTL
- Induces higher avidity CD8 CTL
- Overcomes the need for CD4 T cell help to elicit prolonged CD8 T cell memory
- Is a critical natural mediator by which CD4 T help elicits long-lived CD8 memory T cells

Thus IL-15 is a most promising candidate to enhance the efficacy of vaccines for use in HIV-infected or cancer patients with a deficiency of CD4 T cell help (including therapeutic vaccines for AIDS or cancer).
Use of IL-15 in the vaccine to induce high avidity CTL (recognizing low densities of peptide-MHC complexes on cells)

Improve CTL quality by using IL-15 to substitute for CD4⁺ T cell help to induce long-lived memory CTL

Remove the brakes by blocking negative regulation: A new NKT regulatory axis.
Cancer vaccines can induce CTL measured in vitro but much less often induce clinical tumor regression. 

**WHY?**

**TUMOR TOLERANCE**

- **suppression by tumor** (anti-inflammatory Cytokines/STAT3 induc)
- **suppression by immune cells**
- **absence of danger signals** (incorrect presentation);
  - Off-signals on T cells (e.g. CTLA-4 or PD-1)
- **M2 macrophages or tumor associated macrophages (TAM)**
- **Natural Killer (NK) T cells**
- **CD4+CD25+ T regulatory cells (Treg)**
- Myeloid-derived suppressor cells (MDSC), Granulocyte suppressors
NKT cells

Unlike NK cells, they express a TCR, but have unusual restriction to a nonclassical MHC molecule.
NKT cells and IL-13 suppress CTL tumor immune surveillance though the IL-4R-STAT6 pathway to induce TGF-β production by CD11b⁺Gr-1⁺ cells.

NKT cells

NKT CELLS ARE A HETEROGENEOUS CELL POPULATION

<table>
<thead>
<tr>
<th></th>
<th><strong>Type I</strong></th>
<th><strong>Type II</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>or Classical NKT cells</td>
<td>or Non-classical NKT cells</td>
</tr>
<tr>
<td>CD1d-dependent</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Glycolipid specificity</td>
<td>Alpha-GalCer, OCH</td>
<td>Sulfatide</td>
</tr>
<tr>
<td>TCR-α chain</td>
<td>Vα14-Jα18 (in mice)</td>
<td>diverse</td>
</tr>
</tbody>
</table>

A new immunoregulatory axis

Infected cells

- lysis

NK

- lysis

PD-1

CTL

CTLA-4

promotion of immunity

suppression of immunity

cross-regulation

Treg

type I NKT

CD1d

APC

type II NKT

CD1d

APC

Terabe & Berzofsky, Trends in Immunol, 2007
PUSH-PULL Approach to Optimizing Vaccine-induced T-cell Immunity

Optimize Antigen
(e.g. epitope enhancement)

Cytokines (e.g. IL-15)
Costimulatory molecules
Toll-like receptor ligands
(e.g. CpG, Poly I:C)

Quantity increase
Quality improvement

PUSH
(and steer)

Optimize Immune Response

Block negative regulation (PULL)

Negative Regulators
(e.g. Treg, reg NKT, CTLA-4, PD-1, TGF-β)
Key Collaborators

• Antibody-inducing Adeno-HER-2/neu vaccine: Jong-Myun Park, Masaki Terabe, Jason Steel, Yoshio Sakai, Guido Forni, John Morris

• Epitope enhancement: Jeff Ahlers, Takahiro Okazaki, Pablo Sarobe, SangKon Oh, Ira Pastan

• IL-15: SangKon Oh, Tom Waldmann, Liyanage Perera, Masaki Terabe, Don Burke

• Negative Regulation: Masaki Terabe, Elena Ambrosino, Jong Myun Park, Susanne Ostrand-Rosenberg, Mark Smyth, Dale Godfrey, Vipin Kumar, Takashi Yamamura