A promising candidate to enhance vaccine efficacy against cancer and HIV: IL-15

Jay A. Berzofsky, M.D., Ph.D.
Vaccine Branch, CCR, NCI

National Cancer Advisory Board Meeting
Bethesda, November 30, 2006
The cast and the questions

- **Infected cell**
- **Cancer cell**
- **CD8+ Killer T cell** (Cytotoxic T lymphocyte Or CTL)
- **CD4+ Helper T cell**
- **Dendritic Cell (DC)**

Requirements: 1. High avidity 2. Long-lived memory

Role of IL-15?

Requirement for “help” to get long-lived memory killer T 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
What is the role of IL-2 and IL-15 in the induction of immune response and the maintenance of immunity?

vPE16

vPE16/IL-15

vPE16/IL-2

Primary

Boost

Day 0

3 wks

14 months

Frequency of antigen specific CD8 T cells
Cytolytic activity

vPE16 is a recombinant vaccinia virus expressing HIVgp160
Immunization with antigen + IL-15 induces higher avidity memory CD8\(^+\) CTL

Oh et al., PNAS 2004

2 months after immunization
Hypothesis: are high avidity CTL more effective at viral clearance?

High Avidity CTL Clear Virus Infection in SCID Mice More Effectively Than Low Avidity CTL

Alexander-Miller et al., PNAS 1996
Only HIGH AVIDITY CTL kill tumor cells

Immunization with antigen + IL-15 induces higher avidity memory CD8+ CTL

Oh et al., *PNAS* 2004

2 months after immunization
Higher avidity cells express more IL-15Rα, proliferate more in vivo, and persist longer.

Proliferation 3 wk after adoptive Transfer to naïve Recipients.

Oh et al., PNAS 2004
IL-15 increases the expression levels of CD8β in high avidity CD8+ CTL, and further increases their avidity

Oh et al., PNAS 2004
Role of IL-15 and costimulation in CTL Avidity Maturation

IL-15 in vaccine

High Avidity CTL

Increased IL-15Rα

Selection at population level

Induction at individual cell level

Increased survival and Homeostatic proliferation

Increased CD8 αβ Higher functional avidity

Oh et al. J. Immunol., 2003; Oh et al. PNAS, 2003; Oh et al. PNAS, 2004
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
  - TCR
  - CD28
  - MHC Class I
  - B7 costimulator
  - IL-15
  - IL-12

- CD4⁺ Helper T cell
  - TCR
  - CD40L
  - CD40
  - MHC Class II

- Dendritic Cell
  - CD40
  - MHC Class II
  - IL-15 with vaccine
IL-15 during immunization substitutes for CD4$^+$ T cell help to induce long-lived memory CTL
(One year after immunization)

**Numbers of antigen-specific CD8$^+$ T cells**

- vPE16/Undepleted
- vPE16/CD4-depleted
- vPE16-IL-15/CD4-depleted
Helpless CD8 T cells upregulate TRAIL and undergo TRAIL-mediated apoptosis (programmed death) on restimulation.

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

What if knock out the gene for IL-15?
To test whether IL-15 is necessary for such help to be effective, we examined whether effective help required dendritic cells that could produce IL-15.

Wild-type B6 DC

Coat w OVA peptide & helper epitopes

IL-15

IL-15(-/-) B6 DC

Recipients have normal T helper cells and DC making IL-15

Wild-type B6 mice

Read-outs:
Number of specific CTL (tetramer-binding cells)

Killing activity
DC from IL-15/-/- mice fail to induce memory Ova-specific CD8⁺ T cells: Help requires induction of IL-15

% of specific CD8 T cells

Killing activity

Immunization with:
- WT DC
- IL-15(-/-) DC

% of tetramer+ CD8 T cells

Days after immunization

Oh et al., unpublished
Conclusions

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 and prevent TRAIL-mediated apoptosis
- Is a critical natural mediator by which CD4 T help elicits long-lived CD8 memory T cells

Responsiveness to IL-15 also accounts for CTL avidity maturation by
- Selective survival of high avidity CTL
- Induction of expression of CD8 coreceptor

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).
Collaborators

SangKon Oh¹, ²
Perera, L. Para³
Donald S. Burke²
Masaki Terabe¹
Thomas A. Waldmann³
Jay A. Berzofsky¹

¹Vaccine Branch, CCR, NCI, NIH

²Center for Immunization Research, School of Public Health, Johns Hopkins University

³Metabolism Branch, CCR, NCI, NIH
In vivo boosting of CD8 T cells primed without CD4 help upregulates TRAIL, Bax, and Caspase 3. IL-15 in vaccine reduces these and increases Bcl-XL, blocking apoptosis.

Western blot of bead-purified cells

Oh et al., unpublished
CD4+ T helper cells stimulating DC presenting cognate antigen to secrete IL-15

OTIi OVA-Specific CD4 T cells + DC ± OVA --> Assay sup for IL-15

Antigen presented by APCs to OTIi helper cells

- Ova 22 µg/ml
- Ova 2.2 µg/ml
- none

IL-15 production
pg/ml

below limit
IL-15 at priming selects for longer lived, higher avidity memory CTL and IL-15 responsiveness may account for CTL avidity maturation and replace CD4 help

**Background:**

- IL-15 is known to be required for maintenance of CTL, but we asked whether priming in the presence of IL-15 would affect the quality of the response long after the IL-15 was gone.

- We previously showed that high avidity CTL were more effective at clearing virus in vivo, but it was not known how to induce them preferentially with a vaccine.

- T cell avidity maturation has been a longstanding enigma, in that TCRs do not undergo somatic mutation, unlike antibodies.

- CD4⁺ T cell help is necessary for induction of long-lived memory CTL, but the molecular mechanism of this help is unknown.
Improved viral clearance by high avidity CTL

High avidity CTL

Low avidity CTL

IL-15 mimics CD4⁺ T cell help to prevent TRAIL-mediated apoptosis on restimulation of T cells in vitro

Oh et al., unpublished
Hypothesis:

These results may in part explain recent observation from Schoenberger lab (Nature, 2003) and the Shen and the Bevan labs (Science, 2003) that a major role of CD4\(^+\) T-cell help in a CD8\(^+\) T cell response is to induce long-lasting memory T cells.
Concurrent staining of CD8+ CTL with anti-CD8 and tetramer can separate CD8+ CTL bearing a broad range of avidity: *Proliferation assay of the sorted-cells*

Oh et al., *PNAS* 2004
High avidity CD8\(^+\) CTL express higher levels of CD8\(\beta\), but not TCR\(\beta\) (2 months after boosting) (Avidity by Concurrent staining)

Oh et al., *PNAS* 2004
TRAIL expression on antigen-specific tetramer$^+$ CD8$^+$ T cells from immunized mice after restimulation with peptide and APC in vitro:

Higher TRAIL in cells from mice immunized without IL-15, after CD4 depletion

5 days after in vitro restimulation

Oh et al., unpublished
IL-15 substitutes for CD4⁺ T cell help to Induce long-lived memory CTL

Oh et al., unpublished

Oh et al., unpublished

E:T = 50:1

% Specific lysis

Days after immunization

vPE16/ Not depleted
vPE16/CD4-depleted
vPE16-IL-15/ Not depleted
vPE16-IL-15/CD4-depleted

% of Peak Response Remaining

Activity
Tetramer⁺
51% 26%
44% 18%
34% 16%
<10% 1%
(< Bkgd)
High, Intermediate, and Low Avidity CTL Generated by Stimulation with Different Concentrations of Peptide Antigen

Alexander-Miller, Leggatt, & Berzofsky, PNAS 93: 4102, 1996

% Specific Release

Concentration of I10 (nM)

Concentration of I10 to generate line (μM)

- 100
- .1
- .0001

Alexander-Miller, Leggatt, & Berzofsky, PNAS 93: 4102, 1996
IL-2 & IL-15: SHARED FUNCTIONS IN IMMUNE SYSTEM (ADOPTIVE IMMUNITY)

Epithelium
BM Stroma

Macrophage

DC

IL-15

B
Proliferation
IgA

CTL
Effector Function

CD4

CD8
Proliferation

Activated CD4

IL-2
MHC class I tetramer

MHC class I

Peptide

Cell membrane

β₂M

α₁

α₂

α₃

Peptide

β₂M

α₂

α₁

α₃

Peptide

β₂M

α₂

α₂

α₁

α₁

α₂

β₂M

Streptavidin

Biotin

Biotin

Biotin

Fluorescent dye
Higher avidity cells express higher levels of IL-15Rα and proliferate more in response to natural levels of IL-15 (homeostatic proliferation).

Homeostatic Proliferation 3 wk after adoptive Transfer to naïve Recipients.

<table>
<thead>
<tr>
<th>Brightness of tetramer staining</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>87.2%</td>
</tr>
<tr>
<td>Intermed</td>
<td>56.4%</td>
</tr>
<tr>
<td>Low</td>
<td>43.6%</td>
</tr>
</tbody>
</table>

Oh et al., *PNAS* 2004