T cells IV: Cytotoxicity and Cell Death
Peptide Loading on MHC Class I in the ER

What Cells Do When the Blitz is On
Antigen Presentation Pathways:
Two Old:

MHC Class I presentation of peptides
MHC Class II presentation of peptides

and Two New:

Cross-priming of exogenous peptides (MHC Class I)
CD1-mediated presentation of glycolipids

Question: How do viruses that don’t infect “professional APCs” such as dendritic cells elicit a primary immune response? After all, virally-infected cells normally don’t traffic to 2’ lymphoid organs
"Classic" view of CTL response against virus-infected cells

- PVR expressed on non-hematopoietic cells.
- Infection with Poliovirus.
- Endocytosis of virus, nuclear entry, synthesis of viral proteins in cytosol.
- Presentation of viral peptides on MHC Class I to CD8+ cytotoxic T-cells.
- Proliferation of cytotoxic T-cells (CTLs).
- Perforin/granzyme-mediated cell death.

Cross-priming of exogenous antigens by dendritic cells

- PVR expressed on non-hematopoietic cells.
- Infection with Poliovirus.
- Cytopathic changes; recognition and phagocytosis by dendritic cell.
- Phagosome-to-cytosol protein export; ubiquitin-mediated proteolysis of viral proteins; Presentation of peptide via MHC Class I.
- Perforin/granzyme-mediated cell death of DC; proliferation of CD8+ CTL.
- Killing of virus-infected epithelial cells by CTL.

Cross-priming: A Dendritic Cell Engulfs a Virus-infected Macrophage

From: Albert et al., J. Exp. Med. 188:1359, 1998
Question: Does development of the cytotoxic T cell response require “help” from CD4 cells (analogous to help for B cells)?

CD8 T Cells Need Help With Their Memory*

*You will, too, in a couple of years
Memory T-cells Don't Forget

Table 1: Estimated survival of virus-specific T-cell memory after smallpox vaccination

<table>
<thead>
<tr>
<th>Vaccinations</th>
<th>Volunteers with CD4+ T-cell memory*</th>
<th>20-30 years</th>
<th>31-50 years</th>
<th>61-75 years</th>
<th>10+ years of CD4+ T-cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100% (16/16)</td>
<td>89% (70/79)</td>
<td>52% (23/44)</td>
<td>10 (6-17)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>88% (10/12)</td>
<td>78% (29/37)</td>
<td>57% (6/9)</td>
<td>8.3 (0-14.1)</td>
<td></td>
</tr>
<tr>
<td>3-14</td>
<td>82% (23/28)</td>
<td>91% (28/32)</td>
<td>ND</td>
<td>12.4 (40-20.5)</td>
<td></td>
</tr>
</tbody>
</table>

*Percentages of volunteers with virus-specific T-cell memory are based on the proportion of immunized participants with a 100% positive IFN-γ Elispot response to smallpox vaccine, compared to controls. The table provides 100% accuracy at 1 month after vaccination or vaccination and 92-95% specificity, based on the vaccine-induced IFN-γ response in controls. Volunteers with CD4+ T-cells were determined 1 year after the last smallpox vaccination. ESF estimated half-life ± 0.5 in standard 95% confidence interval in parentheses is based on linear regression analysis using data from previous 1 and 24 N.C., not determined.


Differences Between Selected T Cell Subsets

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Naïve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migration</td>
<td>LN, spleen</td>
</tr>
<tr>
<td>Cell cycle</td>
<td>+/+</td>
</tr>
<tr>
<td>Cytokine secretion</td>
<td>-</td>
</tr>
<tr>
<td>Peripheral LN homing (L-Selectin; CD62L)</td>
<td>+++</td>
</tr>
<tr>
<td>Adhesion Molecules (Integrins, CD44)</td>
<td>+</td>
</tr>
<tr>
<td>Chemokine Receptors (partial list)</td>
<td>CCR7</td>
</tr>
<tr>
<td>IL-2 Receptor (CD25)</td>
<td>-</td>
</tr>
<tr>
<td>FasL</td>
<td>-</td>
</tr>
</tbody>
</table>
### Phenotypic Differences Between Selected T Cell Subsets

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Naïve</th>
<th>Effector</th>
<th>Memory</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Central</td>
</tr>
<tr>
<td>Migration</td>
<td>LN, spleen</td>
<td>Inflamed tissue</td>
<td>LN</td>
</tr>
<tr>
<td>Cell cycle</td>
<td>+/-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Cytokine secretion</td>
<td>-</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Peripheral LN homing</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>(L-Selectin; CD62L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adhesion Molecules</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>(Integrins, CD44)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemokine Receptors (partial list)</td>
<td>CCR7</td>
<td>CCR5 CXCR4</td>
<td>CCR7</td>
</tr>
<tr>
<td>IL-2 Receptor (CD25)</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>FasL</td>
<td>-</td>
<td>+++</td>
<td>-</td>
</tr>
</tbody>
</table>

### Effector Memory Cells Can Secrete Cytokines

**Cooperation in Killing:**

*Granzyme and Perforin*

---

### Structure of Perforin

![Structure of Perforin](image)

### Human Diseases Involving Defective Granule Killing*

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene</th>
<th>Clinical Manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chediak-Higashi Syndrome</td>
<td>CHS1</td>
<td>Lysosomal inclusions in all leukocytes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recurrent bacterial infections</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased NK cell function</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oculocutaneous albinism (melanosome defect)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bleeding (platelet storage granule defect)</td>
</tr>
<tr>
<td>Griscelli Syndrome</td>
<td>Rab27a</td>
<td>Partial albinism</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hepatosplenomegaly (lymphohistiocytic infiltration)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased NK cell function</td>
</tr>
<tr>
<td>Hermansky-Pudlak Syndrome</td>
<td>HPS1</td>
<td>Oculocutaneous albinism (melanosome defect)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bleeding (Platelet storage granule defect)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pulmonary fibrosis (Type II cell surfactant body inclusions)</td>
</tr>
<tr>
<td>Familial Hemophagocytic Lymphohistiocytosis</td>
<td>Perforin</td>
<td>Hepatosplenomegaly (accumulation of activated T-cell and macrophages)</td>
</tr>
<tr>
<td></td>
<td>(30% of cases)</td>
<td>Decreased NK cell function</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pan cytopenia</td>
</tr>
</tbody>
</table>

*Do not memorize this list*
Life (and Death) in the Fas Lane


The TNF Superfamily and Selected Immune Functions

- Overlapping structure and function
- Death domain-containing members tend to induce cell death (but not always)
- Signaling via homotrimerization and recruitment of adaptor proteins
- In addition to regulating cell survival, many members participate in co-stimulation

### The TNF Superfamily and Selected Immune Functions

<table>
<thead>
<tr>
<th>Receptor on T cell</th>
<th>Ligand</th>
<th>Immune Function</th>
<th>Disease Associations</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFR1</td>
<td>TNF-α</td>
<td>Apoptosis</td>
<td>RA, IBD, psoriasis</td>
</tr>
<tr>
<td>TNFR2</td>
<td>TNF-β</td>
<td>Apoptosis</td>
<td>RA, IBD, psoriasis</td>
</tr>
<tr>
<td>Fas</td>
<td>Fas-L</td>
<td>Apoptosis</td>
<td>RA, IBD, psoriasis</td>
</tr>
<tr>
<td>RANK</td>
<td>RANKL</td>
<td>Bone resorption</td>
<td>RA</td>
</tr>
<tr>
<td>LTβR</td>
<td></td>
<td>Lymph node development; Th1 responses</td>
<td>many</td>
</tr>
<tr>
<td>CD40L</td>
<td>CD40L</td>
<td>Ig class switching</td>
<td>XLA, viral</td>
</tr>
<tr>
<td>4-1BB</td>
<td>4-1BB</td>
<td>Co-stimulation of memory CD8+ T cells</td>
<td>viral, RA, IBD</td>
</tr>
<tr>
<td>OX40</td>
<td>OX40</td>
<td>Co-stimulation of activated CD4+ T cells</td>
<td>viral, RA, IBD</td>
</tr>
<tr>
<td>BCMA</td>
<td>APRIL</td>
<td>B cell survival</td>
<td>CVID, RA</td>
</tr>
<tr>
<td>TACI</td>
<td>BAFF</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Legend**
- L: Ligand

**Bone resorption (osteoclasts)**
- RA = Rheumatoid arthritis
- IBD = Inflammatory bowel disease
- ALPS = Autoimmune lymphoproliferative syndrome
- XLA = X-linked agammaglobulinemia
- GVHD = Graft-vs-host disease
- CVID = Common variable immunodeficiency


### Viral Evasion of Immunity
**Cross-priming: DCs Fight Back**

- **Infected DC**: Pathogen, Shuts down, No migration.
- **Infected tissue cell**: Pathogen, Cross-presentation into the class I pathway.
- **Cross-presenting DC**: Cross-presentation to CD8+ T cells in the draining lymph node.

**Major Lymphocyte Subpopulations in Peripheral Blood and Selected Effector Functions**

<table>
<thead>
<tr>
<th>B cells</th>
<th>T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>“Other”</td>
</tr>
<tr>
<td>Ab production</td>
<td>Ag presentation</td>
</tr>
</tbody>
</table>
Innate-like Lymphocytes

B cells  T cells

B1  NK, NKT, γδ

Innate-like B Lymphocytes

B-1 cells

- Make natural antibody, protect against infection with *Streptococcus*
- Ligands not MHC associated
- Cannot be boosted
B-1 Cells: B Prepared

Marginal zone B cells, like B-1 cells, respond to carbohydrate antigen and secrete mainly IgM. After the initial encounter of Ag, they demonstrate little memory (like trying to cram this course).

Thymus-independent Antigens are Presented to Specialized B-cells

Marginal zone B cells, like B-1 cells, respond to carbohydrate antigen and secrete mainly IgM.

After the initial encounter of Ag, they demonstrate little memory (like trying to cram this course).
Innate-like T Lymphocytes: NK Cells

<table>
<thead>
<tr>
<th>NK cells</th>
<th>Produce cytokines rapidly</th>
<th>Ligands not MHC associated</th>
<th>Cannot be boosted</th>
</tr>
</thead>
</table>

Natural Killer Cell
How do NK Cells Recognize Their Targets?

The human MHC covers ~4 Mbp of DNA on chromosome 6p21.3 and contains over 220 identified loci. It has been divided into three regions: class II (centromeric), class III, and class I (telomeric) with extended class I and class II regions on either side. This is one of the most gene-dense regions of the human genome. It encodes the most polymorphic human proteins known to date. Of the expressed loci in the MHC, roughly 40% are associated with the immune system. They include the classical class I, HLA-A, B, and C, nonclassical HLA-E, -F, and -G, as well as "postmodern" MICA and MICB genes (MHC class I chain-related genes). The products of classical polymorphic class I genes, HLA-A, B, and C, interact with T cell receptor (TCR) molecules as well as with the products of the killer immunoglobulin-like receptor (KIR) genes expressed on natural killer cells and some T cells.

Trowsdale., Immunity. 15:363, 2001
One Mechanism of Triggering NK Cell Cytotoxicity

The NKG2D-DAP10 receptor complex and its ligands. Cytotoxicity requires the expression of ligands of NKG2D (e.g., MICA, MICB) in the "stressed" target cells.


Why do NK Cells Fail to Recognize Healthy Cells?

MHC class I on normal cells is recognized by killer inhibitory receptors (KIRs) or by lectin-like CD94-NKG2 heterodimers on NK cells, which inhibit signals from activating receptors.

NK cell does not kill the normal cell.

Activated NK cell releases granule contents, inducing apoptosis in target cell.

"Killed" or absent MHC class I cannot stimulate a negative signal. NK cell is triggered by signals from activating receptors.

NK cell activating ligand.

KIR 00

KIR 2D

CD94/NKG2

MHC class I

NK cell activating ligand.

NK cell

NK cell

Activated

Activated
Innate-like T Lymphocytes

Epithelial γδ cells
- Produce cytokines rapidly
- Ligands are MHC class Iβ associated
- Cannot be boosted

NK T cells
- Produce cytokines rapidly
- Ligands are lipids bound to CD1d
- Cannot be boosted
Structure of the CD1b Molecule--
Look Familiar?

Processing of Glycolipid Antigens from
M. tuberculosis by APCs:
I. Phagocytosis and Glycolipid Processing in Endosomes


II. Fusion of Endosomes with CD1d-containing Vesicles

III. Loading of Glycolipids onto CD1d Molecules


Structure of the CD1b Molecule--
Look Familiar?

CD1b with bound glycolipid antigen
Processing of Glycolipid Antigens from *M. tuberculosis* by APCs:

I. Phagocytosis and Glycolipid Processing in Endosomes

II. Fusion of Endosomes with CD1d-containing Vesicles


III. Loading of Glycolipids onto CD1d Molecules

VI. Trafficking of CD1d-glycolipid Complexes to the Surface of the APC


Question: Do lymphocytes of the acquired immune system even care about lymphocytes of the innate immune system?
Innate Immune Lymphocytes Trigger Dendritic Cell Maturation

Summary

1. For cytotoxic CD8 T-cells, ligation of the TCR by MHC I/peptide + co-stimulation results in release of granzymes and perforin and/or FasL, leading to apoptosis of the target cells.

2. Viruses evade host defense, in part, by down-regulating MHC Class I. Uninfected dendritic cells circumvent this by "cross-priming": phagocytosis of virus-infected cell and presentation of "exogenous" viral antigens on MHC Class I.

3. CD8 T cells can function without CD4 help, but need CD4 help to develop into effective memory cells. CD4 memory cells live for years; central memory cells home to lymph nodes and effector memory cells home to inflamed tissue.

4. NK cells lack TCRs, but instead express both activating and inhibitory (e.g., KIRs) receptors at their surfaces. The relative expression and ligation of these receptors determines the outcome (i.e., killing or not) of the NK effector response.

5. Innate immune B-cells (e.g., B-1 cells and marginal zone B cells) recognize carbohydrate antigens, secrete IgM, and are not long-lived.

6. Innate immune T-cells (γδ T-cells, and NK T cells) recognize non-peptide antigens in non-classical MHC-like molecules. They mediate cytotoxicity, rapid cytokine secretion, and trigger maturation of DCs (and therefore initiate acquired immunity).