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Kuby Immunology
SEVENTH EDITION

CHAPTER 13
Effector Responses: Cell- and Antibody-Mediated Immunity

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Fundamental Distinctions Between *Humoral* and *Cell-Mediated* Immune Responses

- recognition of extracellular versus intracellular pathogens
  - cell surface vs. MHC-peptide complexes

- **CMI** primarily effective against:
  - virus-infected cells
  - intracellular bacteria, fungi
  - tumors
  - graft rejection
The Central Players of the Cell-Mediated Immune Response

- **adaptive immunity**: specific (Ag-specific, MHC-restricted)
  - CD8$^+$ CTLs
  - CD4$^+$ T$_h$1 (mediate DTH and recruitment of additional effectors, mainly macrophages, to sites of inflammation)
  - CD4$^+$ T$_h$2 (promote antibody production)

- **innate immunity**: nonspecific
  - lymphoid: NK cells, NKT cells
  - non-lymphoid: macrophages, granulocytes (i.e., neutrophils, eosinophils)
Cell-Mediated Effector Responses
Generation of Effector CD8⁺ CTLs

Sequential

1. Naïve CD8⁺ T cell
2. Class I MHC
3. IL-2

Figure 13-5a
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Generation of Effector CD8⁺ CTL (cont’d)

Simultaneous

Figure 13-5b
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CTL-Tumor Cell Interaction
Steps of CTL-Mediated Target Cytolysis

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Early Stages of the CTL-Target Interaction:
TCR Activation Facilitates Conformational Changes in LFA-1 Which Increase Affinity for ICAM-1
Later Stages of the CTL-Target Interaction: Pore Formation in the Target Cell Membrane
Experimental Evidence for Perforin and Fas Ligand as the Major CTL Effector Mechanisms

(a) Generation of CTLs

Normal H-2^b → Lymphocytes → Mitomycin C → Killed lymphocytes → Normal H-2^b anti-H-2^k CTLs

Normal H-2^k → Lymphocytes → Mitomycin C → Killed lymphocytes

Perforin knockout H-2^b → Lymphocytes → Mitomycin C → Killed lymphocytes

Normal H-2^k → Lymphocytes

(b) Interaction of CTLs with Fas^+ and Fas^- targets

<table>
<thead>
<tr>
<th>CTLs</th>
<th>Target cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal H-2^k</td>
</tr>
<tr>
<td>Normal H-2^b anti-H-2^k</td>
<td>Killed</td>
</tr>
<tr>
<td>Perforin knockout H-2^b anti-H-2^k</td>
<td>Killed</td>
</tr>
</tbody>
</table>

Figure 13-12
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Apoptotic Pathways Triggered by Perforin and FasL-Based Effector Mechanisms
Assays for Detection and Measurement of T Cell Responses
Proliferation as a Measurement of T Cell Response

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Cytotoxicity as a Measurement of T Cell Response

(a) Strain Y cells

Strain X

4–5 days

Spleen cells

Lymphocytes

$^{51}$Cr-labeled strain Y cells

Measure $^{51}$Cr release

(b) LCM virus

Spleen cells

Lymphocytes

Syngeneic $^{51}$Cr-labeled cells infected with LCM virus

Measure $^{51}$Cr release
Phenotypic Analysis of 'MHC-Tetramer'-Reactive Cells as a Measurement of T Cell Response

Figure 13-6
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Tracking Ag-Specific CTLs *In Vivo* Using MHC-Tetramers

Blood and organs

Lung 30%
Peripheral blood 19%
Kidney 41%
Mesenteric lymph node 2.5%
Gut 28%
Bone marrow 7%

Lymphoid tissues and organs
Peripheral lymph node 0.6%
Liver 30%
Spleen 11%
Innate Immunity

NK cells
NKT cells
phagocytes
macrophages
granulocytes
Natural Killer Cells

- activity due to large granular lymphocytes, which comprise 5-10% of PBMC

- important for host defense against certain intracellular pathogens, including bacteria, viruses and tumor cells

- also, important for immune regulation and bridging innate and adaptive immune responses via cytokine production, such as IFN-γ
  - modulate macrophage effector functions
  - influence Th1 commitment

- involved in early phase of infections, which provide ample kinetics for CTL generation; NK cells are stimulated by; e.g., IFN-α, IL-2, IL-12

- lytic mechanisms are similar to those used by CTL
  - perforin pathway is constitutively active
  - death receptor killing
  - cytotoxic cytokines

- immune response is dictated by the balance of positive and negative signals delivered via activating and inhibitory receptors
Model of Cross-Talk Between NK (Innate) and Adaptive Immunity in Response to a Viral Challenge
NK Recognition

- **Activating Receptors (AR)**
  - interact with stress-induced ligands
    - induced on target cells as a result of DNA damage, infection, etc., leading to downregulation of conventional MHC molecules
    - induction of stress-induced ligands in concert with the loss of MHC expression culminates in NK activation

- **Inhibitory Receptors (IR)**
  - interact with MHC molecules
    - signals generated via IR inhibit NK activation
      - under normal conditions of no cell damage
      - under conditions of cell damage if MHC expression is not profoundly compromised
    - IR signals predominate over AR signals
Model for NK Recognition and Lysis of Target Cells: Opposing-Signals Model

(a) Class I MHC Inhibitory receptor
    Normal cell AR engage ligands on target cell surfaces
    Ligand Activating receptor NK cell No killing
    Activation can be circumvented by IR which detect MHC expression levels

(b) Virus-infected cell (class I MHC)
    Ligand Activating receptor NK cell Killing
    NK activity is suppressed if MHC levels are unaltered, thus sparing normal cells
Experimental Design to Test for NK Cell 'Memory'

MCMV-infected mice

- MCMV-infected cell
  - Ly49H

  Naïve natural killer cell
  - NK1.1

  Expansion

Long-lived experienced natural killer cell
- Ly49H

  NK1.1

Contraction

MCMV-infected cell

In vitro

- Ly49H-mediated killing of MCMV infected cells than naïve NK cells.

In vivo

- Protection from MCMV infection

Transfer to immunodeficient newborn mice

Box 13-2 Figure 1
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Evidence for NK Cell ‘Memory’ Response

![Graph showing survival rates of different NK cell populations](image)

- **10^4 memory NK (n=12)**
- **10^5 naïve NK (n=18)**
- **10^4 naïve NK (n=16)**
- **PBS (n=15)**

Days after infection with MCMV

Survival (%)
**Summary:**

**Comparison Between NK Cells and CTLs**

- **Similarities**
  - derived from bone marrow and common early progenitors
  - express shared markers, such as CD2, IL-2Rβ
  - share lytic pathways, including perforin and FasL
  - *evidence for immunologic memory*

- **Differences**
  - do not express Ag-specific TCR or CD3
  - are not MHC-restricted
  - do not require antigenic priming
  - develop extra-thymically: can be found in *nude*, SCID and RAG-deficient mice, all of which lack functional T cells
Summary: Comparison Between NKT Cells and CTLs

• Similarities
  - derived from bone marrow and common early progenitors, including thymic development
  - express conserved elements of the TCR-α/β chains, and some subsets may express CD4
  - thought to be involved in the control of certain bacterial and viral infections, as well as tumors, and does so via cytokines (e.g., TNF-α) or Fas/FasL interactions

• Differences
  - are not conventionally MHC-restricted, but recognize glycolipids presented by a non-polymorphic CD1d molecule
  - currently, no evidence for immunologic memory
  - express markers more characteristic of NK cells than T cells
Summary:
Innate and Adaptive Immune Mechanisms of Antigen Recognition

Helper cells

CD4+ T cell
- TCR
- Peptide

CD8+ T cell
- TCR

NKT cell
- NK1.1
- Glycolipid
- Semi-invariant Vα14–Jα18 TCR

Cytotoxic cells

NK cell
- NK1.1
- Activating NK receptor

Class II MHC

Class I MHC

CD1d

Class I MHC

Viral antigens or tumor antigens

Figure 13-16
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### Summary: Cell-Mediated Effector Mechanisms

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Effector molecules produced</th>
<th>Mechanism of killing</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTL (typically CD8+ T cell)</td>
<td>Cytotoxins (perforins and granzymes), IFN-γ, TNF, Fas ligand (FASL)</td>
<td>Cytotoxic granule release and FASL-FAS interactions</td>
</tr>
<tr>
<td>NK T cell</td>
<td>IFN-γ, IL-4, GMCSF, IL-2, TNF</td>
<td>FASL interactions predominantly; can activate NK cells indirectly</td>
</tr>
<tr>
<td>NK cell</td>
<td>Cytotoxins (perforins and granzymes), IFN-γ, TNF, Fas ligand (FASL)</td>
<td>Cytotoxic granule release and FASL-FAS interactions</td>
</tr>
</tbody>
</table>

Table 13-4

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Antibody-Mediated Effector Functions, Including ADCC
Antibody-Mediated Effector Functions

1. Virus and toxin neutralization
   - Prevents pathogen-host binding

2. Opsonization
   - Phagocytosis

3. Complement fixation and formation of the membrane attack complex
   - Phagocytosis or lysis

4. Antibody-Dependent Cell-mediated Cytotoxicity (ADCC)
   - NK-induced apoptosis

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Figure 13-1
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Signal Transduction Pathways Triggered in Response to FcR Engagement

Macrophage

Activation (including production of cytokines, enhancement of phagocytic activity)
Antibody-Dependent Cellular Cytotoxicity

- NK cells, monocytes/macrophages, neutrophils and eosinophils express Fc receptors reactive with the Fc portion of target cell surface-specific IgG

- multiple mechanisms of lysis, including degranulation, secretion of cytotoxic enzymes/cytokines

![Diagram showing the interaction between immune cells and target cells in antibody-dependent cellular cytotoxicity.]
<table>
<thead>
<tr>
<th>mAb product (trade name)</th>
<th>Nature of antibody</th>
<th>Target (antibody specificity)</th>
<th>Modification of antibody</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rituximab (Rituxan)</td>
<td>Chimeric</td>
<td>CD20 (mouse B-cell antigen)</td>
<td>None</td>
<td>Relapsed or refractory non-Hodgkin's lymphoma</td>
</tr>
<tr>
<td>Trastuzumab (Herceptin)</td>
<td>Humanized</td>
<td>Human epidermal growth factor receptor 2 (HER-2)</td>
<td>None</td>
<td>HER-2 receptor positive advanced breast cancers</td>
</tr>
<tr>
<td>Alemtuzumab (Campath)</td>
<td>Humanized</td>
<td>CD52 (an antigen on many types of leukocytes)</td>
<td>None</td>
<td>Chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>Bevacizumab (Avastin)</td>
<td>Humanized</td>
<td>Vascular endothelial growth factor (VEGF)</td>
<td>None</td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>Cetuximab (Erbitux)</td>
<td>Chimeric</td>
<td>EGFR</td>
<td>None</td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>Panitumumab (Vectibix)</td>
<td>Human</td>
<td>EGFR</td>
<td>None</td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>Ibritumomab</td>
<td>Mouse</td>
<td>CD20</td>
<td>None</td>
<td>Relapsed or refractory non-Hodgkin's lymphoma</td>
</tr>
<tr>
<td>Ibritumomab tiuxetan (Zevalin)</td>
<td>Mouse</td>
<td>CD20</td>
<td>$^{90}Y$</td>
<td>Relapsed or refractory non-Hodgkin's lymphoma</td>
</tr>
<tr>
<td>Tositumomab (Bexxar)</td>
<td>Mouse</td>
<td>CD20</td>
<td>$^{131}I$</td>
<td>Relapsed or refractory non-Hodgkin's lymphoma</td>
</tr>
<tr>
<td>Gemtuzumab ozogamicin (Mylotarg)</td>
<td>Humanized</td>
<td>CD33 (glycoprotein antigens on myeloid progenitor cells and monocytes)</td>
<td>Attached to an anti-tumor agent that cleaves double-stranded DNA at specific sequences</td>
<td>Acute myelogenous leukemia</td>
</tr>
<tr>
<td>Epratuzumab</td>
<td>Humanized</td>
<td>CD22 (glycoprotein antigens on mature and neoplastic B cells)</td>
<td>None</td>
<td>Relapsed or refractory non-Hodgkin's lymphoma</td>
</tr>
</tbody>
</table>