Lecture: T Cell Activation and Regulation

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Lecture Overview

• Anatomical concerns

• “The rules of engagement”
  - T cell activation requires more than the generation of foreign peptide-self MHC complexes on APC’s.....

• T cell signaling

• Two signal model and co-stimulation (bulk of the lecture)

• Putting it all together
The life history of T lymphocytes

Precursors mature in the thymus

Naïve CD4+ and CD8+ T cells enter the circulation

Naïve T cells circulate through lymph nodes and find antigens

T cells are activated and develop into effector and memory cells

Effector T cells migrate to sites of infection

Eradication of infection
Principles of lymphocyte activation

- Lymphocytes are normally in a resting state in lymphoid organs and circulation

- Rapid response to antigen (activation) --> proliferation, change to functionally active and diverse populations

- Migration to tissues, where they perform their function of eliminating infections

- Multiple possible steps for therapeutic targeting
Functional responses of T lymphocytes

Activation of naive T cells in lymph node, development of effector cells

Activation of effector T cells at site of infection; eradication of microbe
Steps in the activation of T lymphocytes.

- **Antigen recognition**
  - Naive CD4+ T cell
  - APC
  - Naive CD8+ T cell

- **Activation**
  - IL-2R
  - Cytokines (e.g., IL-2)

- **Clonal expansion**
  - Effector CD4+ T cell
  - Memory CD4+ T cell
  - Effector CD8+ T cell (CTL)

- **Differentiation**
  - Activation of macrophages, B cells, other cells
  - Killing of infected "target cells"; macrophage activation

**Lymphoid organs**

**Peripheral tissues**
Kinetics of a T cell response

Number of antigen specific T cells (x 10^6)

- Naive
- Clonal expansion
- Effector response
- Decline (homeostasis)
- Memory

Days post-infection

From: Abbas & Lichtman, Cellular & Molecular Immunology, W. B. Saunders, 2003
Signals for T cell activation

• **Antigen recognition**
  - Regulated movement of signaling receptors and adhesion molecules at (immune synapse)

• **Costimulators** (second signals)

• **Cytokines**
  - Produced by APCs or T cells
  - Stimulate T cell expansion and differentiation into effector cells
The recognition of a peptide-MHC complex by a T cell antigen receptor.
Antigen recognition by T cells

- Each T cell sees an MHC molecule and bound peptide
  - Dual recognition determines specificity and MHC restriction
- Each T cell sees very few (1-3) residues of the peptide antigen
  - T cells distinguish between diverse microbes based on recognition of few amino-acids
- The affinity of TCR-antigen interactions is low
  - Kd on the order of $10^{-5}$ to $10^{-6}$
  - Because T cells are selected by recognition of self MHC in the thymus (the only MHC they can encounter during their lives)
  - T cell-APC contacts need to be stabilized by other molecules
- The activation of T cells may require multiple or prolonged TCR-antigen interactions
  - T cell receptors and signaling proteins assemble in the synapse
Proposed models to reconcile T cell sensitivity to Ag

- Oligomerisation
- Membrane microdomains (rafts)
- Immunological synapse
- Binding in two-steps of the TCR to its ligand
  
  (sampling of MHC-peptide complexes at the surface of APCs)
T cells first “stick” to APC's using cell adhesion molecules

![Diagram](image-url)
TCR’s scan for MHC-peptide complexes and if present can promote adhesion through a conformational change in LFA-1.

The role of integrins in the adhesion of T cells to APCs.

- Weak adhesion → no T cell response
- TCR or chemokine mediated increase in integrin affinity
- Strong T cell–APC adhesion → T cell response

Integrin activation

Integrin (low affinity state)

Ligand for integrin

APC

Chemokine

Chemokine receptor

TCR

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T cells use co-receptors for antigen recognition.
Formation of the immunological synapse

Regulated way of bringing together key signaling molecules
Functions of the immune synapse

• Promote signaling
• Terminate signaling: recruitment of phosphatases, ubiquitin ligases, inhibitory receptors (e.g. CTLA-4) to the site of the TCR complex
• Direct effector molecules to the relevant target: cytokines, CD40L, perforin, etc
initial association via CDR1 and CDR2 (on rate)
- induces fitting of the CDR3 loops on the peptide (off rate)
- stabilize the interaction

allow an efficient scanning of the surface of Ag presenting cell to detect foreign peptide (rare and very similar to self) in a very sensitive manner
Two-steps binding of the TCR to its ligand

- MHC residues (α helices) affect association (guide the TCR to its ligand)
- allow conformational change of the CDR3 loops
- peptide residues affect the dissociation or stability of the tri-molecular complex

**T cell receptor-induced signal transduction pathways**

- **Initiation of TCR-mediated signals**
  - Biochemical intermediates
    - Increased cytosolic Ca^{++}
    - Diacylglycerol (DAG)
  - Active enzymes
    - Calcineurin
    - PKC
    - ERK, JNK
  - Transcription factors
    - NFAT
    - NF-κB
    - AP-1

From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 8-8
TCR signalling is dynamically regulated

- Csk and CD45 are continually phosphorylating and dephosphorylating Lck
- Phosphorylation of Lck inhibits its activation activity
- When TCR stimulation occurs PAG1 is dephosphorylated and Csk is released thus removing the inhibitory phosphorylation of Lck
TCR signalling is dynamically regulated (cont).

- Cbl family proteins are Ubiquitin Ligases that tag phosphorylated adaptors for destruction in the lysosome.
- When Cbl-b is knocked out, mice develop a severe autoimmune syndrome highlighting the importance of the termination of signaling.
Initial responses to activation

- #1 rule: key cytokine the T cell needs to make is IL-2

- Proliferation. Mostly dependent on IL-2 through an autocrine pathway.

- Other cytokines, cytokine receptors will also get produced and lead to effector T cell development (lecture upcoming...).
Resting T cells express only a moderate-affinity IL-2 receptor (IL-2Rβ and γ chains only)

Activated T cells express a high-affinity IL-2 receptor (IL-2Rα, β and γ chains) and secrete IL-2

Binding of IL-2 to its receptor signals the T cell to enter the cell cycle

IL-2 induces T-cell proliferation

Figure 8-20 Immunobiology, 6/e. (© Garland Science 2005)
Steps in the activation of T lymphocytes.

1. **Antigen recognition**
   - Naive CD4+ T cell
   - Naive CD8+ T cell

2. **Activation**
   - IL-2R
   - Cytokines (e.g., IL-2)

3. **Clonal expansion**
   - Effector CD4+ T cell
   - Effector CD8+ T cell (CTL)

4. **Differentiation**
   - Memory CD4+ T cell
   - Memory CD8+ T cell

5. **Effector functions**
   - Activation of macrophages, B cells, other cells
   - Killing of infected "target cells"; macrophage activation

**Lymphoid organs**

**Peripheral tissues**
The Two-Signal Model of T-cell Activation

1+2=Full activation
Two signal requirement for lymphocyte activation

- Naïve lymphocytes need two signals to initiate responses
  - Signal 1: antigen recognition
    - Ensures that the response is antigen-specific
  - Signal 2: microbes or substances produced during innate immune responses to microbes
    - Ensures that the immune system responds to microbes and not to harmless antigenic substances
    - Second signals for T cells are “costimulators” on APCs and cytokines produced by APCs
Marc Jenkins and Ronald Schwartz in the late 80’s:
The first definitive experimental demonstration that TCR engagement alone was insufficient for T cell activation.

Proliferative response of T cell clones
(pigeon cytochrome c peptide 81-104 presented by I-E^k)
to normal or ECDI(chemical crosslinker)-fixed peptide-pulsed APCs

ECDI-treated APCs fail to stimulate proliferation by normal T cell clones:
Not the result of extensive modification of the MHC class II molecule
ECDI treatment inactivated an accessory (costimulatory) function of the APC.
The role of costimulation in T cell activation.

**Antigen recognition**

- "Resting" (costimulator-deficient) APC
- Activation of APCs by microbes, innate immune response
- Activated APC: increased expression of costimulators, secretion of cytokines
- Cytokines (e.g., IL-12)

**T cell response**

- Naive T cell
- IL-2
- Effector T cells
- T cell proliferation and differentiation

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The concept of costimulation was consistent with the characteristics of a newly identified molecule called CD28, which is expressed on naive CD4+ and CD8+ T lymphocytes.

**Artificial APC**

- Ag/MHC
- TCR
- CD28

**Physiological APC**

- Ag/MHC
- TCR
- CD28

Stimulating Ab

Blocking Ab (FAb’2)

**T cell**

- TCR
- CD28

**Stimulating Ab**

**Blocking Ab (FAb’2)**

**Protein**

- Beads

**Protein**

- Stimulation

- IL-2 production

**Protein**

- Prevention

- Induction of anergy

**Protein**

- Proliferation

- IL-2 production
### The B7:CD28 families

<table>
<thead>
<tr>
<th>Expression</th>
<th>DCs, macrophages, B cells</th>
<th>DCs, macrophages, B cells</th>
<th>DCs, macrophages, B cells, other cells</th>
<th>DCs, macrophages, B cells, other cells</th>
<th>DCs, lymphoid and nonlymphoid cells</th>
<th>DCs, lymphoid and nonlymphoid cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>B7-1 (CD80)</td>
<td>B7-2 (CD86)</td>
<td>ICOS-L</td>
<td>PD-L1 (B7-H1)</td>
<td>PD-L2 (B7-DC)</td>
<td>B7-H3</td>
</tr>
</tbody>
</table>

#### Ligands on APCs and other cells

#### Receptors on T cells

- **CD28**: T cells; constitutive
- **CTLA-4**: T cells; inducible
- **ICOS**: T cells; inducible
- **PD-1**: T cells, B cells, Myleoid cells; inducible

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<tr>
<th>Name</th>
<th>CD28</th>
<th>CTLA-4</th>
<th>ICOS</th>
<th>PD-1</th>
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<td>T cells; constitutive</td>
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<td>T cells, B cells, Myleoid cells; inducible</td>
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<tr>
<td><strong>Major function</strong></td>
<td>Costimulation of naive T cells; generation of regulatory T cells</td>
<td>Negative regulation of immune responses; self-tolerance</td>
<td>Costimulation of effector T cells</td>
<td>Negative regulation of T cells</td>
<td>Costimulation or negative regulation of T cells</td>
<td>Negative regulation of T cells</td>
</tr>
</tbody>
</table>

Selected B7-CD28 family members

- B7-1, B7-2 (CD80/CD86)
- ICOS-L
- B7-1, B7-2 (CD80/CD86)
- PD-L1/PD-L2

APC membrane

T cell membrane

- CD28
- ICOS
- CTLA-4
- PD-1

ITIM motif
ITSM motif
Tyr-X-X-Met

+ + - -
Activation of T cells by peptide-pulsed DCs in vivo: requirement for B7

DO11 T cells (Ova-specific TCR transgenic) labeled with CFSE and transferred into normal or B7-knockout recipients ---> immunized with Ova peptide-pulsed cultured dendritic cells from normal or B7-knockout recipients ---> response of DO11 cells assayed
Memory cells are less dependent on B7 costimulation than are naïve T cells.

### Naïve CD4 T cells

- **Proliferation (CPM)**
- **Antigen (µg/ml)**

### Memory CD4 T cells

- **Proliferation (CPM)**
- **Antigen (µg/ml)**

**APCs**
- **wild type (normal; positive control)**
- **B7.1/2-/-**
- **None (negative control)**
B7:CD28 dependence of T cells

• Initiation of T cell responses requires B7:CD28

• Dependence on B7-CD28:
  - Naïve > Th1 > Th2 > memory
  - CD4 > CD8
  - Regulatory T cells
CD28 Signals through its cytoplasmic tail SH2-binding sites. A major downstream signaling enzyme is PI3 kinase.
TCR and CD28 Signaling cooperate to help promote IL-2 production
## Major effects of CD28-mediated costimulation in T cells

<table>
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<tr>
<th>Proliferation</th>
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<tbody>
<tr>
<td>IL-2 (transcription, mRNA stabilization)</td>
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<tr>
<td>IL-2R up-regulation</td>
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<tr>
<td>↑G1 cell cycle kinases</td>
</tr>
<tr>
<td>↓Cell cycle inhibitor p27Kip</td>
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<th>Survival</th>
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<tbody>
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<td>Bcl-xL</td>
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<tr>
<th>Effector function</th>
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<tr>
<td>↑ CD40-L, OX-40, 41BB, ICOS</td>
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<tr>
<td>↑ cytokines expression</td>
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<tr>
<td>↑ cytotoxic molecules</td>
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The major effects of CD28-mediated costimulation are to augment and sustain T cell responses initiated by antigen receptor signal by promoting T-cell survival and enabling cytokines to initiate T cell clonal expansion and differentiation.
TCR and CD28 dissociate over time in the cSMAC

Youosuka et al. 2008, Immunity
Are there unique pathways for CD28 signaling?

Liang et al. 2013, Nature Immunology
CD40L is upregulated on T cells after initial priming. This causes APC's to further upregulate B7 ligands.
The opposing functions of CD28 and CTLA-4

- Knockout of CTLA-4 results in autoimmune disease and loss of normal homeostasis:
  - multi-organ lymphocytic infiltrate, lethal by 3-4 weeks
  - lymphadenopathy, splenomegaly
CTLA-4 – Master regulator of T cell activation
T cell inhibition by CTLA-4

Compete for B7

- APC
- T cell
- CD28
- CTLA-4

Block signaling

- T cell
- TCR
- CTLA-4
- CD28
- MHC-pep
- B7
Cytokine production by primed T cells

**IL-2**

**IFN-γ**

**IL-4**

- **DO.11/wild-type**
- **DO.11/CTLA-4**
The inhibitory functions of CTLA-4

- **Role in self-tolerance:**
  - Autoimmunity and lymphoproliferation in knockout mice
  - Polymorphism associated with autoimmune diseases in humans
  - Blockade or deletion makes T cells resistant to tolerance, exacerbates autoimmune diseases (EAE, type 1 diabetes)
How does CTLA-4 regulate T cell function

CTLA-4 Binds
- JAK2
- SHP-2
- PP2A catalytic subunit
- AP-2 (endosome sorting)
- Associates with TCRζ
- Engagement results in dephosphorylation of
- Proximal TCR signals - TCRζ, ZAP-70, LAT

No Antigen

Antigen

CTLA-4 binds to TCR/CD3, leading to downstream targets and dephosphorylation of proximal TCR signals, resulting in clonal unresponsiveness.
Both CD28 and CTLA-4 go to the Immunological synapse

Expression of B7-1 and/or B7-2 on the APC is not required to induce redistribution of CD28 or CTLA-4 upon contact with a T cell.

TCR signaling is required

Egen and Allison
(Immunity Jan. 2002)
The opposing actions of CD28 and CTLA-4

CD28 and CTLA-4 both recognize B7-1, 2; yet CD28 stimulates and CTLA-4 inhibits

- **Kinetics:** CD28 is expressed constitutively and initiates responses; CTLA-4 appears later and terminates responses

- **Affinity:** CD28 binds to B7 only when B7 levels are high (microbes?), CTLA-4 (high affinity) binds when B7 is low (self antigens?)

- **Preferential ligands:** CD28-->B7-2 (constitutive); CTLA-4-->B7-1 (inducible)
Mechanisms of homeostasis in immune responses (T cells)

- **T cell expansion**
  - Activated T cells express CTLA-4
  - Activated T cells are deprived of antigen and other stimuli
- **Functional inactivation**
- **Apoptosis**
  - Surviving memory cells

From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 10-14
B7h/ICOS costimulatory pathway

A new molecule with structural characteristic similar to the B7 molecules was identify in 1999, and was named \textbf{B7h} (B7-related protein 1; also GL-50 or B7RP-1 or ICOS-L).

B7h does not bind to CD28 or CTLA-4, but bind to \textbf{ICOS} (inducible costimulatory molecule). ICOS shares 30-40\% sequence similarity with CD28 and CTLA-4.

\textbf{ICOS expression:}
ICOS is not constitutively expressed on naïve T cells but is \textit{induced} on CD4+ and CD8+ T cells following stimulation through the TCR and is further enhanced by CD28-mediated costimulation.

\textbf{FIGURE 2.} Expression of ICOS on activated T cells. Dissociated splenocytes from wild-type or B7-1/2/-129/SvS4Jae mice were incubated with anti-CD3, anti-CD3 and CD28, or no Ab. The thick line shows ICOS expression on T cells from wild-type splenocyte cultures, the dotted line shows ICOS expression on T cells from B7-1/2/- splenocyte cultures, and the thin line represents a negative staining control (rat IgG-FITC).

Antibody response and germinal center formation in ICOS -/- mice

ICOS is required for antibody responses and GC formation.

The PD-1 inhibitory pathway

- PD-1 recognizes two ligands (PD-L1, PD-L2)
- Upregulated on T cells after activation
- Knockout of PD-1 leads to autoimmune disease (different manifestations in different strains)
- Role of PD-1 in T cell suppression in chronic infections?
In chronic LCMV infection in mice, virus-specific T cells become paralyzed; express high levels of PD-1; function restored by blocking the PD-1 pathway. Barber et al (Ahmed lab) Nature 2006
Roles of inhibitory receptors

- Maintenance of self-tolerance
- Immunosuppression in chronic infections (HCV, HIV?)
- Termination of normal immune responses?
- Why so many inhibitory pathways?
Putting it back together
Context matters: APC's upregulate B7 upon recognition of microbes.
Figure 8-14 Immunobiology, 6/e. (© Garland Science 2005)
Anatomy of naïve T cell priming—APC's

Antigen uptake by Langerhans' cells in the skin

Langerhans' cells leave the skin and enter the lymphatic system

Langerhans' cells enter the lymph node to become dendritic cells expressing B7

B7-positive dendritic cells stimulate naïve T cells

Figure 8-15 Immunobiology, 6/e. (© Garland Science 2005)
Anatomy of naïve T cell priming (cont.)

- **Figure 8-4 Immunobiology, 6/e. (© Garland Science 2005)**

**T cells enter lymph node across high endothelial venules in the cortex**

- T cells that do not encounter specific antigen leave lymph node through lymphatics

- T cells monitor antigen presented by macrophages and dendritic cells

- T cells that encounter specific antigen proliferate and differentiate to effector cells
In vivo T cell activation
Mempel et al. Nature 2004

In vivo imaging of T cells adoptively transferred into mice with antigen loaded DC’s

DC’s are red and T cells are green

Observed three phases of T cell behavior:

Phase 1: multiple short encounters with DC’s

Phase 2: long-lasting stable contacts with DC’s

Phase 3: resumed short contacts and rapid migration
Movies
What does movie mean?
Are T cells scanning?
When are synapses forming?
Mechanisms of homeostasis in immune responses (T cells)

- **T cell activation**
  - Activated T cells express CTLA-4
  - Activated T cells are deprived of antigen and other stimuli

- **T cell expansion**
  - CD28
  - B7

- **Functional inactivation**
- **Apoptosis**
- **Surviving memory cells**

From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 10-14
After T cell activation, differentiation into other subsets
Summary

- TCR-MHC/peptide interaction is low affinity. T cells use multiple mechanisms to overcome this (anatomy, adhesion, synapse, etc.)
- Context of MHC-antigen is critical to outcome
- Balance of positive and negative signals determine the magnitude and nature of T cell responses
- Challenges:
  - Which signals are dominant in vivo under different conditions?
  - How do we use this knowledge to design therapeutic strategies?