Cytokines and T cell Signaling

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Overview of TCR signaling

[Diagram showing TCR signaling pathway]
Overview of TCR signaling

Brownlie & Zamoyska, Nature Review Immunology; 2013; Vol 13 p257-269
Overview of TCR signaling

Spatial temporal control of LAT

Brownlie & Zamoyska, Nature Review Immunology; 2013; Vol 13 p257-269
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Spatial temporal control of LAT

Brownlie & Zamoyska, Nature Review Immunology; 2013; Vol 13 p257-269
Overview of TCR signaling

Function of PLC
- Generates IP₃ to induce release of Ca²⁺ from the endoplasmic reticulum into the cytoplasm
- Ca²⁺ works with DAG to activate PKC
Function of PLC

Biophysical properties of the Calcium Release Activated Channel (CRAC)
Calcium signaling in T cells

Once activated, how is expansion of T cell subsets regulated?

Abbas, Lichtman, Pillai. Cellular and Molecular Immunology, 7th edition. Copyright 2012 by Saunders
JAK-STAT

- Janus kinase-signal transducer and activator of transcription
- STAT (Signal Transducer and Activator of Transcription)

Th1 Differentiation
Roles of TNF in T cells

O’Shea, Nature Review Immunology; 2002; Vol 2 p37-45
Roles of TNF in T cells

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Th1 Differentiation

Cytokines and helper T cells

O’Shea, Nature Review Immunology; 2002; Vol 2 p37-45
Inflammatory Cytokines?

- Pro-Inflammatory?
  - IL-2
  - IFN-γ
  - TNF-α

- Anti-Inflammatory?
  - IL4
  - IL10
  - TGF-β
Pro-survival Cytokines?

- Pro-survival?
  - IL-7-survival by not cell cycle entry
  - IL-4-survival by not cell cycle entry
  - IL-2

SURVIVAL VS PROLIFERATION

The Cell Cycle and its Phases
SURVIVAL VS PROLIFERATION

Cell Proliferation Application Decision Tree

- Intact cells
  - Flow cytometry
    - Live
      - BD Horizon™
      - Violet ProFluor™
      - 7-AAD and PI
      - Treg suppression assay
      - Functional assay
      - Cell cycle markers
      - Phosphorylations as indicators of cell proliferation
      - CD45/CD8 expression
  - Fixed
    - BrdU incorporation
    - Ki-67, p-Histone H3, PCNA, cyclins, RB, cytokines

- Cell extracts
  - Bioimaging/microscopy
    - Fixed
      - Phosphorylations as indicators of cell proliferation
      - Ki-67, p-Histone H3, cyclins, etc.

- Tissue sections
  - Bioimaging/immunohistochemistry
    - Ki-67, p-Histone H3, cyclins, etc.
    - Cell cycle markers

Human PBMCs were stimulated with anti-CD3/CD28 for 48 hours and re-stimulated with PMA-Ionomycin for 4 hours, and BrdU was added for the final 1 hour. Cells were then harvested and stained using the BrdU staining protocol.
Traditional view of Cytokines

Revised view of Cytokines

O’Shea, Nature Review Immunology; 2002; Vol 2 p37-45
Skewing of the immune response

- Th1
- Th2
- Th17
- Th0

Expansion of $T_h1$ cell Cells

Yes, but how do we turn off the signaling?

SOCS
- 8 family members **Suppressor of cytokine signaling**
- Induced by cytokine
- Negative feedback loop in the cytokine induced JAK-STAT pathway
- Likely function of E3 ubiquitin ligases and mediate protein degradation of signaling complexes
- SOCS interacts with the MTOC
- Interacts directly through kinase inhibitory region (KIR) to inhibit JAK2-mediated phosphorylation of STAT1
Role of SOCS in T cell development

- May prevent inadvertent signaling from pro-survival cytokines and harmful effects of IFN-γ

Yoshimura, Naka, Kubo, Nature Review Immunology Vol 7 June 2007

Role of SOCS in T cell regulation

- Th1 5-fold higher levels of SOCS1
- Th2 23-fold higher levels of SOCS3

Yoshimura, Naka, Kubo, Nature Review Immunology Vol 7 June 2007
Role of SOCS in T cell regulation

Suppression of $T_h^2$ cells by $T_h^1$ cells

$T_h^1$: 5-fold higher levels of SOCS1
$T_h^2$: 23-fold higher levels of SOCS3

What happens when signals wane?
Cytokine Regulation of metabolism—when growth factors are limited

- Autophagy
- Breakdown of intracellular constituents to generate ATP

Development of Memory T cells

<table>
<thead>
<tr>
<th>Naive T cell</th>
<th>Effector T cells</th>
<th>Memory T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45RA+</td>
<td>CD45R0+</td>
<td>CD45RO+</td>
</tr>
<tr>
<td>CD25lo</td>
<td>CD25hi</td>
<td>CD25lo</td>
</tr>
<tr>
<td>CD127hi</td>
<td>CD127lo</td>
<td>CD127hi</td>
</tr>
<tr>
<td>CD44lo</td>
<td>CD44hi</td>
<td>CD44hi</td>
</tr>
</tbody>
</table>

Abbas, Lichtman, Pillai. Cellular and Molecular Immunology, 7th edition. Copyright 2012 by Saunders
Development of Memory T cells

**T\(_{H2}\) Cells**

- Produce IL-4 stimulates IgE Ab production
- Produce IL-5 which activates eosinophils
- Stimulates phagocyte-independent, eosinophil mediated immunity
  - usually against intracellular infections (parasites)
- IL-4, IL-10 or IL-13 inhibit macrophage activation and suppress T\(_{H1}\) cell mediated response (return to in Chapter 6)

**Disorders affecting lymphocytes**

March 18, 2014
Charlotte M. Vines, Ph.D.
Objectives

- Non malignant disorders
  - Leukocyte adhesion deficiency type II
  - Bare lymphocyte syndrome
  - Bruton’s tyrosine kinase
- Malignant disorders

Thymus
Thymus

Lymph Node
Adaptive Immune response

Diseases due to adaptive immune deficiencies

- X-linked agammaglobulinemia-BTK
- ADA Deficiency
- DiGeorge Syndrome
- Common gamma chain deficiency
- BLSII
- RAG1 or RAG2 deficiencies (missense= Omenn Syndrome)
- Null mutation in RAG1 or RAG2 (SCID)
  - Note SCID is fatal early in life unless immune system is reconstituted-
  - Bone marrow transplants
  - IVIG (B cell defects)
  - Treat patients with antibiotics as needed
Antibody locus

Diseases due to adaptive immune deficiencies - B-cells

- Hyper IgM
- Hyper IgE (Job’s)
- Selective IgA deficiency
- Common variable immunodeficiency
- Wiskott Aldrich Syndrome
Diseases in infants

- Erythroblastosis fetalis
- Transient hypogammaglobulinemia of infancy
- Neonatal Neutropenia

Protective Effect of Maternal Antibodies in Serum and Milk
Autoimmune Diseases

- Rheumatoid arthritis
- Systemic Lupus Erythematosus
- Goodpasture’s syndrome
- Myesthenia gravis

Cancer

- Multiple myeloma
- Leukemias
- Lymphomas
Infection
- HIV-Acquired immune deficiency
- Septic shock
- Toxic shock
- Crohn's

Transplant
- Hyperacute rejection (immediate)
- Acute rejection (weeks)
- Chronic rejection (months- years)
- Graft-vs-host disease
Case

Which of these patients has normal peripheral blood T lymphocytes?

Case

Which of these profiles would you expect in a patient with HIV/AIDS if you examined the peripheral blood T lymphocytes?
Simultaneous zinc-finger nuclease editing of the HIV coreceptors ccr5 and cxcr4 protects CD4+ T cells from HIV-1 infection

2014 123: 61-69

W. Doms

Danet-Desnoyers, James L. Riley, Phillip D. Gregory, C