CD4+ T Cells and Types 1, 2, 3 Immunity

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Lecture outline

• Overview of T cell responses and T cell mediated immunity
• Discovery and definition of Th subsets
• Functions of subsets and roles in human disease
• Additional considerations: hybrid subsets, plasticity, non-Th sources of types 1, 2, 3 cytokines
• Therapeutic targeting of subset cytokines
Types of T Cell–Mediated Immune Reactions

**CD4+ helper T cells (Th)**
- Microbes that live inside phagocytes
- Microbes that are readily killed by phagocytes

**Phagocytes with ingested microbes in vesicles**
- CD4+ effector T cells (Th1 cells)

**Cytokine secretion**
- Macrophage activation ⇒ killing of ingested microbes
- Inflammation, killing of microbes

**CD8+ Cytotoxic T lymphocytes (CTL)**
- Microbes that live inside tissue cells

**Infected cell with microbes or antigens in cytoplasm**
- CD8+ T cells (CTLs)

**Killing of infected cell**
Steps in a CD4+ Helper T Cell Response

Changes in chemokine- and S1P-receptor expression

- CXCR3
- S1PR1
- CCR7

Antigen recognition and induction of response in lymphoid organs

T cell proliferation and differentiation

Differentiated CD4+ Th cells enter circulation

Migration of effector T cells and other leukocytes to site of antigen

Neutrophils and monocytes

CD4+ effector T cells

Effector functions of T cells

Phagocytosis and killing of microbes

Inflammation, leukocyte activation

Microbe

Tissue site of infection

Dendritic cell

Chemokines, cytokines

Neutrophils and monocytes
Cytokine-Mediated Functions of CD4+ Helper T Cells

Activate B cells to produce antibodies which eliminate extracellular microbes

Promote differentiation of CTL which kill infected cells

All this done by one cell type? or Are there subsets of helper T cells with different functions?

Activate macrophages to kill phagocytosed microbes or repair tissues

Promote migration and activation of inflammatory cells
CD4+ Helper T cell subsets: Definitions and Properties

• Populations of CD4+ T cells that make restricted and non-overlapping sets of cytokines

• Distinct functions, migration properties, roles in disease

• Can be identified by gene or protein expression of:
  o Cytokines
  o Trafficking receptors
  o “Master” transcriptional regulators
# Subsets of CD4+ Th Cells

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CD4 Effector T Cell Subsets

**Naïve CD4 T cell**
- **Th1**
  - Migrate to sites of infection and inflammation
  - Elimination of microbes
- **Th2**
- **Th17**
  - Migrate to sites of infection and inflammation
- **Follicular helper T cells (Tfh)**
  - Remain in lymphoid organ, migrate into follicles
  - Help B cells to produce high-affinity antibodies

Lymph node
Differentiation of Th Subsets

Different subsets develop from uncommitted naïve CD4+ T cells

Each subset is induced by the types of microbes that subset is best able to combat

Cytokines produced at the site of antigen recognition drive differentiation into one or the other subset

Sources of cytokines that drive differentiation: APCs, responding T cells themselves, other host cells
**JAK-STAT Cytokine Signaling**

**JAKS**
- Jak1
- Jak2
- Jak3
- Tyk2

**STATS**
- STAT1
- STAT2
- STAT3
- STAT4
- STAT5 (A and B)
- STAT6

**Diagram:**
- Cytokine binding to receptor leads to JAK-mediated phosphorylation of receptor chains.
- Recruitment of STATs to cytokine receptor.
- JAK-mediated phosphorylation and dimerization of STATs.
- Translocation of STATs to nucleus.
- STAT-binding sequences in promoter.
- Transcription of cytokine-responsive gene.
The Functions of Th1 Cells: Macrophage Activation

Activation of macrophages

- APC
- Naive T cell
- Th1 cell
- Bacteria
- IFN-γ

Responses of activated macrophages

- Enhanced killing of phagocytosed bacteria
- Secretion of inflammatory cytokines
- Increased expression of molecules required for T cell activation

Classical macrophage activation (enhanced microbial killing)

CD40L=CD154
Human $T_{H1}$ cells: defense against intracellular microbes

- **Mendelian susceptibility to mycobacterial disease (MSMD):** inborn errors of IFN-$\gamma$ mediated immunity
  - Mutations in genes encoding IL-12 receptor, IL-12, IFN-$\gamma$ receptor, IFN-$\gamma$ signaling molecules
- Most common infections are with microbes that can live inside phagocytes: *Mycobacteria, Salmonella*
- **IL-12 and IFN-$\gamma$ required for Th1 differentiation; IFN-$\gamma$ required for Th1 function (macrophage activation)**
Functions of T_{\text{H}17} cells

Bacteria → APC → Naive CD4+ T cell

Proliferation and differentiation → T_{\text{H}17} cells

T_{\text{H}17} cells

IL-17 → Leukocytes and tissue cells → Chemokines, TNF, IL-1, IL-6, CSFs → Inflammation, neutrophil response

IL-22 → Tissue cells → Anti-microbial peptides → Epithelial repair
STAT3-dependent cytokines in Th17 differentiation

What is the function of human $T_H^{17}$ cells?
...required for defense against extracellular microbes

- Human Stat3 mutations result in HIES*, characterized by infections, as well as many other clinical manifestations
  - Recurrent staphylococcal abscesses or mucocutaneous candidiasis
- HIES patients have impaired $T_H^{17}$ responses.
- Supports role for $T_H^{17}$ cells in resistance to extracellular bacterial and fungal infections

*Hyper-IgE Syndrome, aka Job’s syndrome

Milner JD et al
Nature 452, 773-776. 2008
What is the function of human $T_H^{17}$ cells?
...required for defense against extracellular microbes

CMC: Chronic mucocutaneous candidiasis
(defective anti-fungal immunity)

Genes:
Dectin-1, CARD9
Th17 biology (IL-17F, IL-17RA, IL-17RC, ACT1, STAT3, RORγt)

STAT1 hyperactivation

Cytokine autoantibodies

What are the functions of human Th1 vs. Th17 cells?

- Mendelian susceptibility to mycobacterial disease (MSMD): inborn errors of IFN-γ immunity.
- Some genes involved: IL-12Rβ1, IFN-γR1, IL-12p40, IFN-γR2, STAT-1, IRF8.
- Most common infections with deficiencies in IFN-γR and STAT1: 
  *BCG, environmental mycobacteria, M. tuberculosis, Salmonella*
- Most common infections with deficiencies of IL-12p40, IL-12Rβ1: 
  *Mycobacteria, Salmonella, Candida*

Why both intracellular and extracellular infections in IL-12p40 and IL-12Rβ1 deficiencies?

- p40 shared by IL-12 and IL-23
- IL-12Rβ1 shared by both IL-12R and IL-23R

- IL-12 needed for Th1 differentiation
- IL-23 needed for Th17 differentiation

*Implications for mAb drug therapies*
Functions of T\textsubscript{H}2 Cells

- **IL-4**, **IL-13**
- **IL-25**
- **IL-33**
- **TSLP**

**B cell**
- IL-4, IL-13
- IgG4 (human), IgG1 (mouse)
- Antibody production

**Th1 cell**
- IgE

**Th2 cell**
- IL-4, IL-13
- Eosinophil activation

**Macrophage**
- IL-4, IL-13
- Alternative macrophage activation (tissue repair)

**Mast cell degranulation**
- Intestinal mucus secretion and peristalsis

**Helminth**
Macrophage Activation: Classical & Alternative

Classically activated macrophage (M1)
- Microbial TLR-ligands
- IFN-γ
- ROS, NO, lysosomal enzymes
- IL-1, IL-12, IL-23, chemokines
- Microbicidal actions: phagocytosis and killing of many bacteria and fungi
- Inflammation

Alternatively activated macrophage (M2)
- IL-10, TGF-β
- IL-13, IL-4
- Anti-inflammatory effects
- Tissue repair

Th1
Th2

Tumor killing
Tumor growth
Th differentiation:
Summary of Cytokines and Transcription Factors Involved

Intracellular infections
Parasite infections
Extracellular fungal and bacterial infections
Microbes outside cells

CD4+ T cell
Naive
Activated

DC
CD28

T-bet (Stat 1, Stat 4)
Th1
IFN-γ
IL-12R

IFN-γ
IL-12
IL-25/IL-33
IL-21
TGF-β
ICOS

IL-4
IL-17RB
IL-17F
IL-21
IL-22

RORγt (Stat3)
Th17
Th1

GATA-3 (Stat6)
Th2
IL-4
IL-5
IL-13

Bcl6
ICOS

B

IFN-γ
IL-12R

IL-4
IL-17RB
IL-17F
IL-21
IL-22

IL-6

IL-23R

IL-24

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The real story about Th subsets is more complicated!

- **Additional subsets related to classic subsets**
- **Other sources of the same helper cytokines besides CD4+ Th cells**
- **CD4+ Th cells that blur Th 1, 2, 17 distinctions**
- **Plasticity of Th subsets**
Th cells that make both IL-17 and IFN-γ are important in defense and disease

- Bi-allelic loss-of-function mutations in RORC (encodes RORγT) result in candidiasis and mycobacteriosis#

- Patients lack IL-17A/F-producing T cells (expected)…explains candidiasis

- Patients also have impaired IFN-γ response to mycobacterium (unexpected). IFN-γ production is impaired in:
  - γδ T cells
  - Th1* (a.k.a “nonclassic Th17”) subset: αβ TCR, CD4+ T bet+ RORγT+ IFN-γ+, IL-17A+ CCR6+, CXCR3+

Dual IFNγ/IL-17 producing Th cells (Th1*) may be the major pathogenic effectors in many diseases

- Can be derived from already differentiated classic Th17 cells in response to IL-23
- More abundant than Th1 or Th17 at sites of inflammation in mouse model diseases (EAE) and human diseases (Crohn’s, atherosclerosis)

# see: Burkett PR, Meyer zu Horste G, Kuchroo VK. J Clin Invest. 2015;125:2211-9
Homeostatic and Pathogenic Cells Related to Th17 cells

Common regulatory module: IL-10, coinhibitory molecules, AHR and so on

IL-27 and others

TH1
T-BET
IFNγ

IL-17A
IL-17F
IFNγ
GM-CSF

IL-23
IL-1β

Common proinflammatory module: BHLHE40, GM-CSF, IFNγ, CXCR6 and so on

Homeostasis

Inflammation

Alexandra Schnell 1,2, Dan R. Littman3,4 & Vijay K. Kuchroo 1,2 TH17 cell heterogeneity and its role in tissue. Inflammation. Nature Immunology Review 2023
- No T cell or B cell antigen receptors
- Activated by cytokines
- Effector functions mediated by cytokines
- Subsets analogous to helper T cell subsets
- Present in tissues before infection—contribute to early cytokine responses in host defense and inflammatory diseases

Other sources of the same helper cytokines besides CD4+ Th cells

Innate Lymphoid Cells
Types 1, 2, 3 cells beyond Th’s

Type 1

- CD4+ T-bet
- IL-12R
- CXCR3

Type 2

- INKT GATA-3
- IL-17R
- CXCR3

Type 3

- γδ T T-bet
- IL-12R
- CXCR3

Type 1

- ILC1 T-bet Foxp3
- IL-12R
- CXCR3

Type 2

- ILC2 T-bet Foxp3
- ST2
- IL-17R
- CCR8?

Type 3

- ILC3 RORγt Foxp3
- ST2
- IL-23R
- CCR6

(Th17)

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Non-Th17 Sources of IL-17 in Inflammatory Diseases

- γδ T cells: Psoriasis
- CD8+ T cells: Psoriasis
- Neutrophils: Arthritis, Dermatitis
- iNKT cells: Various
- ILCs: Inflammatory bowel disease

Anti-IL-17 therapy would theoretically apply to all of these
Types 1, 2 and 3 Immunity Model based on Target cells

- **Type 1 cells** that primarily activate and attract mononuclear phagocytes such as monocytes, macrophages and DCs (Th1, ILC1, NK, CTL)

- **Type 2 cells** targeting B cells, mast cells, basophils, and eosinophils (Th2, TH9, ILC2)

- **Type 3 cells** acting on non-hematopoietic cells at barrier tissue sites, including epithelial cells and stromal cells (Th17, ILC3, Th22, CD8 T cells)

Orbital model based on Th cell targets

mAb Targeting Type Type 1, 2, and 3 Diseases

Type 1
- IFN-\(\gamma\): Primary HLH (Emapalumab)

Type 2
- IL-5: Eosinophilic asthma (Reslizumab, Mepolizumab)
- IL-13: Atopic dermatitis (Tralokinumab)
- IL-13/4 receptor: Atopic dermatitis (Dupilumab)

Type 3
- IL-17A: Psoriasis, RA, Ankylosing spondylitis (Secukinumab)
- IL-17RA: Psoriasis, Psoriatic arthritis (Brodalumab)
- IL-23p19 Psoriasis (Guselkumab)

Type 1 and 3
- IL-23 and IL12 p40: Psoriasis, Psoriatic arthritis (Ustekinumab)
Remarkable advances in the treatment of many inflammatory diseases have been achieved using monoclonal antibodies (mAbs) that block the production or action of cytokines or cytokine receptors.

- **Plaque psoriasis (PS)** is a good example of one of those diseases.
- In PS Type 3 responses, dependent on IL-23 and characterized by IL-17, TNF, and IL-22-mediated inflammation, are dominant.
- Type 1 responses with interferon gamma production also contribute.
- mAbs targeting IL-23, IL23R, and IL-17 are now in wide use to treat PS.
JAK inhibitors (JAKinibs)

Jakinibs are small molecule inhibitors of JAKs that block ATP binding to the enzymes’ active sites

Each Jakinib developed has a different range of specificities for the different JAKs

Specificities of Jakinibs in clinical use: pan JAK, JAK1/JAK2, JAK1
JAK inhibitors (JAKinibs)

- Ruxolitinib (JAK1/2)
- Tofacitinib (JAK1/2/3)
- Baricitinib (JAK1/2)
- Upadacitinib (JAK1)

Plaque PSA

Deucravacitinib (Tyk2)

RA

- Ruxolitinib (JAK1/2)
- Tofacitinib (JAK1/2/3)
- Baricitinib (JAK1/2)
- Upadacitinib (JAK1)
- Tofacitinib (JAK1/2/3)

UC

AD

- Upadacitinib (JAK1)
- Abrocitinib (JAK1)