CD4+ T Helper T Cells....
and their cytokines in immune defense and disease

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Types of T Cell–Mediated Immune Reactions

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

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

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

CD4+ effector T cells
(Th17 cells)

Cytokine secretion

Macrophage activation
killing of ingested microbes

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

Killing of infected cell

Inflammation, killing of microbes
Sequence of events in T cell responses

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

**Lymphocyte activation**
- IL-2R
- Cytokines (e.g., IL-2)
- Naive CD4+ T cell
- Naive CD8+ T cell

**Proliferation**
- Effector CD4+ T cell
- Effector CD8+ T cell (CTL)

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

**Effector functions**
- Memory CD4+ T cell
- Memory CD8+ T cell

**Chemokine and S1P receptor expression**
- CXCR3
- S1PR1
- CCR7

**Lymphoid organ**

**Peripheral tissue**

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**Induction of CD4+ Helper T Cell Response**

**Antigen recognition**
- Antigen recognition in lymphoid organs

**Induction of response**
- B7, CD28
- CD4+ effector T cells

**T cell expansion and differentiation**
- Changes in chemokine- and S1P-receptor expression
- Naive T cell

**Differentiated effector T cells enter circulation**
- Naive CD4+ T cells
- CD4+ effector T cells
**Effector Phase of CD4+ T Cell Responses**

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

**Effector functions of T cells**
- Phagocytosis and killing of microbes
- Inflammation, leukocyte activation

**Cytokine-Mediated Functions of CD4+ Helper T Cells**

Activate B cells to produce antibodies which eliminate extracellular microbes

Promote differentiation of CTL which 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

B lymphocyte

Cytotoxic T lymphocyte

Helper T lymphocyte

Macrophage

Granulocyte

Eosinophil

Monocyte/macrophage

Secreted antibody
CD4+ Helper T cell subsets: definitions and properties

- Populations of CD4+ T cells that make restricted and non-overlapping sets of cytokines
  - Early after activation, T cells can produce multiple cytokines
  - Progressive activation leads to "polarization": production of selected cytokines

- Distinct functions, migration properties, roles in disease

### Major Subsets of CD4+ Helper T Cells

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**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
- Major sources of cytokines that drive differentiation: APCs, responding T cells themselves, other host cells

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**Differentiation of Th subsets**

**INDUCTION**
- Cytokines act on antigen-stimulated T cells to induce the transcription of cytokine genes that are characteristic of each subset

**COMMITMENT**
- Epigenetic changes maintain subset cytokine genes in active state, and the T cell becomes committed to one specific pathway.

**AMPLIFICATION**
- Cytokines produced by any given subset promote the development of that subset and inhibit differentiation toward other CD4+ subpopulations.
Development of $T_H1$ Cells

**EFFECTOR FUNCTIONS:**
Macrophage activation

**JAK-STAT CYTOKINE SIGNALING**

**JAKS**
Jak1, Jak2, Jak3, Tyk2

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

Diagram showing the interaction between JAKs and STATs in cytokine signaling.
The canonical Jak–STAT pathway

Development of Th2 Cells

Effectors Functions:
- Alternative macrophage activation
- Mucus production
- Increased gut motility
- Eosinophil activation
Development of Th17 Cells

STAT3-dependent cytokines in Th17 differentiation

Th differentiation:
Summary of Cytokines and Transcription Factors Involved

- **Naïve CD4+ T cell**
  - IL-4
  - IL-12

- **TH1**
  - IFN-γ
  - IL-12
  - STAT 1, STAT 4

- **TH2**
  - IL-4
  - IL-5
  - IL-13
  - STAT 6

- **TH17**
  - IL-17
  - IL-21
  - IL-22
  - STAT 3

Th subsets express distinct sets of chemokine receptors which dictate specific recruitment patterns

- **CXCR3**
  - CXCL 9, 10, 11
- **CCR5**
  - CCL 3, 4, 5
- **CCR4**
  - CCL 22, 17
- **CCR8**
  - CCL 1
- **CCR6**
  - CCL 2, 4, 5, 17, 22

Adapted from: F Annunziato, C Romagnani, and S Romagnani J Allergy Clin Immunol 2015;135:626-35
The Functions of Th1 Cells

Macrophage Activation by TH1 Cells (1)
What is the function of human $T_H1$ cells?

*required for defense against intracellular microbes*

- **Mendelian susceptibility to mycobacterial disease (MSMD):** inborn errors of IFN-$\gamma$-mediated immunity.
- Genes involved: $[\text{IL}-12\beta1, \text{IFN}-\gamma R1, \text{IL}-12p40, \text{IFN}-\gamma R2, \text{STAT}-1]$
- Most common infections with deficiencies in IFN-$\gamma R1$, IFN-$\gamma R2$: *Mycobacteria, Salmonella*
- Most common infections with deficiencies in IL-12$\beta$1, IL-12p40: *Mycobacteria, Salmonella, Candida*

**IL-12** required for Th1 differentiation

**IFN$\gamma$** required for Th1 differentiation and function (mac activation)

**STAT-1** required for IFN-$\gamma$R signaling

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**Functions of $T_H2$ Cells**

[Diagram showing pathways involving $T_H2$ cells and their functions: antibody production, mast cell degranulation, intestinal mucus secretion and peristalsis, eosinophil activation, and alternative macrophage activation (tissue repair)]
Macrophage Activation: Classical & Alternative

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

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

Functions of $T_{H}17$ cells

- APC
- Naive CD4+ T cell
- Proliferation and differentiation
- $T_{H}17$ cells

- Chemokines, TNF, IL-1, IL-6, CSFs
- Anti-microbial peptides
- Inflammation, neutrophil response
- Epithelial repair

- Bacteria
- Leukocytes and tissue cells
What is the function of human TH17 cells? 
...required for defense against extracellular microbes

- Human Stat3 mutations result in HIES*, which includes infection susceptibility, as well as many other clinical manifestations.
  - recurrent staphylococcal abscesses or candidiasis
- HIES patients have impaired TH17 responses.
- Supports role for TH17 cells in resistance to extracellular bacterial and fungal infections

*Hyper-IgE Syndrome, aka Job’s syndrome

What is the function of human TH17 cells? 
...required for defense against extracellular microbes

- Autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy (APECED)* syndrome is a rare autoimmune disease associated with severe chronic mucocutaneous candidiasis (CMC)
- Anti-IL-17A, IL-17F, and IL-22 autoantibodies found in up to 90% of cases, strongly associated with CMC

* a.k.a Autoimmune polyendocrine syndrome type 1 (APS-1); due to AIRE (Auto immune regulator) mutations
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

Signaling pathways that regulate differentiation of human CD4+ T cells into effector subsets

Ma et al. JEM 2-17
Microbes Drive Differentiation of the T_H Subsets Needed for their Defense

- **Th1**
  - Intracellular microbes (mycobacteria)
  - NK cell
  - IL-12
  - IFN-γ
  - Th1 cell

- **Th2**
  - Helminths, mast cells, eosinophils
  - IL-4
  - GATA-3
  - Th2 cell

- **Th17**
  - Extracellular fungi, bacteria
  - IL-1, IL-6, IL-23, TGF-β
  - Th17 cell

Development of Memory T cells

- Thymic output
- Naive T cells
- Memory T cells

% Blood T cells vs Age (Years)

- Naive T cells
- Memory T cells
- Thymic output

- Naive T cell
- Effector T cells
- Memory T cells
- Apoptotic cells
- Uncommitted T helper subset phenotypes
Properties of Memory T cells

- Defining properties: survive in a quiescent state after antigen is eliminated and to mount larger and more rapid responses to antigens than do naive cells.
- Memory cells express increased levels of anti-apoptotic proteins, which may be responsible for their prolonged survival.
- The number of memory T cells specific for any antigen is greater than the number of naive cells specific for the same antigen.
- Memory cells undergo slow proliferation, and this ability to self-renew may contribute to the long life span of the memory pool.
- The maintenance of memory cells is dependent on cytokines but does not require antigen recognition.
- Memory cells are able to migrate to peripheral tissues and respond to antigens at these sites.
- Both CD4+ and CD8+ memory T cells are heterogeneous and can be subdivided into subsets based on their homing properties and functions: Central Memory (in SLOs) and Effector Memory (in mucosal tissues).
- Some memory T cells persist in peripheral tissues for very long periods (Tissue Resident Memory cells, TRM).

The real story is more complicated!

- Additional subsets related to classic subsets (Th9, Th22, Tfh).
- 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.
CD4+ Th subsets: Cellular targets
(Tfh is one more)

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Global overview of T helper cell differentiation
**Innate lymphoid cells (ILCs)**

Cells that produce the same cytokines as subsets of helper T cells but do NOT express TCRs and do not recognize MHC-associated peptide antigens.

**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
**Th cells that make both IL-17 and IFN-γ are important in defense and disease**

- Bi-allelic loss-of-function RORC (encode RORγt) mutations result in candidiasis and mycobacteriosis*

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

- Patients have impaired IFN-γ response to mycobacterium (unexpected)
  
  In these patients 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+

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**Type 1 vs. Type 3 immune defense is not simply attributable to distinct Type 1 vs. Type 3 cellular effectors**

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**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)

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* see: Burkett PR, Meyer zu Horste G, Kuchroo VK. J Clin Invest. 2015;125:2211-9
Factors that alter the intestinal microenvironment can affect Th17 differentiation into pathogenic Th1* cells

Signals and nuclear factors driving Th17 differentiation and pathogenicity
Plasticity of CD4+ T cell subsets

- Cytokine reporter mice show significant plasticity of fully differentiated Th cells.
  - e.g. Th 17 cell can be converted to Th1 cells by stimulation with IL-12 or IL-23, in the absence of TGFβ.
  - Treg can be converted to Th17 cells
- Histone modification studies that mark active/poised vs. silenced promoters of the lineage-determining transcription factors* (e.g. Tbet, GATA3, RORγT) show evidence for subset plasticity
- Much evidence of plasticity exists for human Th cells
- **Th17 cells are the most plastic**

*Trimethylation of lysine 4 on histone H3 (H3K4me3) is a permissive mark
  Trimethylation of lysine 27 on histone H3 (H3K27me3) is a mark of gene silencing.
Targeting Type Th1 (Type 1) /Th17 (Type 3) Responses

- IL-17A: Psoriasis, RA, Ankylosing spondylitis (Secukinumab)
  Type 3
- IL-17RA: Psoriasis, Psoriatic arthritis (Brodalumab)
  Type 3
- IL-23 and IL12 p40 Psoriasis, Psoriatic arthritis
  (Ustekinumab)
  Type 1 and 3
- IL-23p19 Psoriasis (Tildrakizumab Phase III)
  Type 3
- IL-6R inhibitors approved for RA are theoretical inhibitors of
  Th17 differentiation

Anti-Cytokine mAb Approved 2017

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<th>Target</th>
<th>Indication first approved</th>
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<tr>
<td>Dupilumab</td>
<td>IL-4Rα</td>
<td>Atopic dermatitis</td>
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<tr>
<td>Sarilumab</td>
<td>IL-6R</td>
<td>Rheumatoid arthritis</td>
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<td>Guselkumab</td>
<td>IL-23 p19</td>
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<tr>
<td>Benralizumab</td>
<td>IL-5Rα</td>
<td>Asthma</td>
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Therapeutic targeting of theJak–STAT pathway

Approved Jak inhibitors (‘jakinibs’)

Tofacitinib, JAK1 and JAK3 inhibitor; approved for rheumatoid arthritis, in trials juvenile arthritis, psoriasis, alopecia areata, ankylosing spondylitis, lupus and ulcerative colitis14

Ruxolitinib, a JAK1 and JAK2 inhibitor approved for treatment of polycythemia vera and myelofibrosis

Oclacitinib, a JAK1 and JAK2 inhibitor, approved for dermatitis in dogs

Targets of drugs approved and in trials

References


