Advances toward understanding the Th17 pathway in IBD

Casey T. Weaver, M.D.
University of Alabama at Birmingham

IFNγ and IL-17 are differentially expressed by intestinal CD4 T cell subpopulations:
Which CD4 T cells mediate colitis?

Balb WT (no colitis)

Balb IL-10-/- (colitis)
**Divergence of the CD4 effector T-cell lineages**

- **Tn** → **Ag**
  - IL-12R1
  - TGF-β
  - IL-6
  - IL-21
  - IL-12R1
  - IL-23R
  - Th17
  - IL-17(A)
  - IL-17F
  - IL-22
  - IFN-γ
  - Neutrophilic Inflammation
  - Microbicidal
  - Bacteria
  - Fungi
  - IL-12Rb1
  - IL-23R
  - STAT3
  - IL-1R
  - Th1
  - IL-18R
  - Monocytic Inflammation
  - Intracellular Killing
  - Bacteria
  - Protozoans
  - Viruses
  - IFN-γ
  - Th2
  - IL-4
  - IL-5
  - IL-13
  - Eosinophilic
  - Basophilic Inflammation
  - Mucosal Clearance
  - Helminthes

**Reciprocal pathways in Th17 vs iTreg development**

- Commensals?
- Type 17 Pathogens (bacteria, fungi)
- Commensals?
- αt-RA
- TGF-β
- IL-21
- IL-6
- IL-21
- IL-12R
- IL-23R
- STAT3
- IL-1R
- Th17
- IL-17A
- IL-17F
- IL-22
- IL-8
- G-CSF
- Antimicrobial peptides
**Effector Sites**

- Villus
- Enterocytes (IECs)
- Lamina propria
- Enteric bacteria
- Crypt
- Naive CD4 T cells
- Mesenteric lymph node
- Vascular circulation
- Teffectors

**Inductive Sites**

- Peyer's patch
- Isolated lymphoid follicle
- Enteric lymphatics

**Il17f/Thy1.1 knock-in reporter mouse model**

**Il17f/a Gene Cluster**

- chr. 1

**Il17f Gene**

- Exon 1
- Exon 2
- Exon 3
- CDS
- UT

**Targeted Il17f allele**

- Exon 1
- Exon 2
- Exon 3
- CDS
- UT

**Post germline neo excision**

- 5' probe ( Hind III)
- 3' probe (BamHI)

**Gene Cluster**

- Il17f
- Il17a
- Chr. 1

**Neo r**

- IRES
- LoxP site

**Camara**

- 5' probe (Hind III)
- 3' probe (BamHI)
**Il17f/Thy1.1 knock-in reporter mouse model**

- Reporter expression is lineage-specific
- Expression of Thy11 (IL-17F) is restimulation-independent, expression of IL-17A is restimulation-dependent
- IL-17A+ cells largely are a subset of IL-17F+ cells in early development

---

**Cytokine-driven divergence in late Th17 development**

Naïve Il17f/Thy1.1 CD4 T cells

- p40-deficient APCs + anti-CD3
- TGF-β, IL-6 (5 d)

FACS Sort

Pre-sort

Thy1.1-

Thy1.1+

Lee et al., Immunity (in press)
**Th1 vs Th17 transcriptome signatures**

Reciprocal Maintenance or Extinction of Th17 and Th1 Lineage Factors by TGF-β or IL-12
**In vivo conversion of Th17 cells assoc. w/ colitis**

![Diagram of cell conversion](image)

**Late Divergence in Th17 Lineage; Antagonism between TGF-β and IL-12**

![Diagram of Th17 lineage](image)
Summary/Conclusions

• Th17 cells demonstrate substantial late-stage developmental plasticity resulting in divergent cytokine and functional phenotypes; Th17p give rise to IL-17A or IFN-γ producers (or both) contingent on prevailing cytokines.

• There is differential retention of IL-17A and IL-17F expression in vivo: IL-17F largely transient; subpopulation retains IL-17A.

• Th17 cells retain responsiveness to IL-12 and transition rapidly to ‘Th1’-like phenotype with loss of IL-17A/IL-17F, gain of IFNγ expression (STAT4/T-bet-dependent); IL-23 also drives divergence of population, but distinct “Th1s”.

• Relative role of IL-17A and IFNγ producers in IBD pathogenesis unclear; both can be derived from Th17 precursors and may be pathogenic.
**The Th1-Th17 Autoimmunity Puzzle**

Experimental autoimmune encephalomyelitis (EAE)
Collagen-induced arthritis (CIA)
Multiple models of experimental colitis
Experimental autoimmune uveitis (EAU) assoc. with:
- Elevated IFNγ
- Elevated IL-12
- Treatment efficacy of anti-IL-12p40 mAb
- Passive transfer of Th1 cells
- Disease ablation in IL-12p40-, STAT4- and T-bet-deficient mice

**The Th1-Th17 Autoimmunity Puzzle**

HOWEVER:
- IFNγ- and IFNγR-deficient mice remain disease-susceptible
- STAT1-deficient mice susceptible
- IL-12p35-deficient mice susceptible
Why CD4 T cells as principal mediators of IBD?

- CD4 T cells recognize antigenic epitopes processed from extracellular antigens that load the MHC class II pathway (e.g., bacterial antigens)
- CD4 T cells orchestrate innate immune cell actions through cytokine and cell contact-dependent networks: modulate innate immune cell pro- (e.g., IFN-γ, IL-17, CD40L) or anti- (e.g., IL-10) inflammatory cytokine production, as well as development/recruitment of innate immune cells (e.g., IL-17)
- CD4 T cells give rise to long-lived memory cells capable of recognizing commensal antigenic epitopes in perpetuum

Timeline of CD4 Effector T-cell Lineage Discoveries

- 1974: T cells "help" Ab & DTH responses
- 1978: T1/T2 model proposed
- 1986: Innate/adaptive immunity Linked (IL-12)
- 1987: T1/T2 distinct Ab Isotypes
- 1988: IL-17 identified
- 1992: IL-23 cloned
- 1993: T17 distinct lineage
- 2000: TGFβ- IL-6 induces T17
- 2003: T1/T2 from naive precursors
- 2005: Autoimmunity linked to IL-23/IL-17 axis
Interleukin 12 Family

IL-12

IL-23

IL-27

STAT4

STAT3

STAT1

Cytokine receptor homology domain
Ig domain
Fibronectin-like domain
Four helix bundle cytokine

**IL-17 Cytokine Family**

- Disulfide-linked homodimers (30–35 kd)
- Five receptors; unique structural family
- Pro-inflammatory effects in multiple tissues/disease states


---

**IL-23 stimulates IL-17 production by memory, but not naïve, CD4 T cells**

Naïve CD4 T cells
(CD44\text{lo}/CD62L\text{hi})

IL-23

Memory CD4 T cells
(CD44\text{hi}/CD62L\text{lo})

IL-17A
IL-17F

Aggarwal/Gurney, JBC 278:1910 (2003)
**Major CD4 T cell lineages**

- **Th1**
  - IFN\(_\gamma\)
  - IL-12
  - IL-4
  - IL-1β
  - IL-17A
- **Th2**
  - IL-5
  - IL-13
- **Th17**
  - IL-17F
  - IL-22
- **Foxp3\(^+\) IT\(_R\)**
  - IL-10
  - TGF-β
  - IL-35
- **Foxp3\(^+\) Tr1**
  - TGF-β
  - IL-10
  - IL-35

**Effector T Cells**

**Summary/Conclusions**

- Th17 cells demonstrate late-stage developmental plasticity.
- Retention of IL-17A/IL-17F expression is TGF-β-dependent.
- Th17 cells retain responsiveness to IL-12 despite relatively low levels of IL-12Rβ2 expression; transition rapidly to 'Th1'-like phenotype with loss of IL-17A/IL-17F, gain of IFN\(_\gamma\) expression (STAT4-dependent).
- IL-23 can also drive transition to IFN\(_\gamma\) expression, but distinct from IL-12 (eg, IL-22, IL-24); more strongly antagonized by TGF-β.
Collaborators

UAB
Dan Bullard
Chuck Elson
Robin Hatton
Pat Higgins
Bob Kesterson
Tom Ryan
Trenton Schoeb
Tim Townes

Duke University
Greg Crawford

LIAI
Hilde Cheroute

NCI
Don Court

Support: NIH NIAID & NIDDK

<table>
<thead>
<tr>
<th>Family member</th>
<th>Other common names</th>
<th>Receptors</th>
<th>Main functions</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-17A</td>
<td>IL-17 and CTLA8</td>
<td>IL-17RA and IL-17RC</td>
<td>Autoimmune pathology, neutrophil recruitment and immunity to extracellular pathogens</td>
<td>T₈, T₁₇ cells, CD8⁺ T cells, CD8⁺ T cells, NK cells, NKT cells and LTi cells</td>
</tr>
<tr>
<td>IL-17B</td>
<td>NA</td>
<td>IL-17RB</td>
<td>Pro-inflammatory activities?</td>
<td>Cells of the gastrointestinal tract, pancreas and neurons</td>
</tr>
<tr>
<td>IL-17C</td>
<td>NA</td>
<td>IL-17RE</td>
<td>Pro-inflammatory activities?</td>
<td>Cells of the prostate and fetal kidney</td>
</tr>
<tr>
<td>IL-17D</td>
<td>NA</td>
<td>Unknown</td>
<td>Pro-inflammatory activities?</td>
<td></td>
</tr>
<tr>
<td>IL-17E</td>
<td>IL-25</td>
<td>IL-17RA and IL-17RC</td>
<td>Induces T₈, T₁₇ cell responses and suppresses T₈, T₁₇ cell responses</td>
<td>Intestinal lymphocytes, lung epithelial cells, alveolar macrophages, eosinophils, basophils, NKT cells, T₈, T₁₇ cells, mast cells, and cells of the gastrointestinal tract and uterus</td>
</tr>
<tr>
<td>IL-17F</td>
<td>NA</td>
<td>IL-17RA and IL-17RC</td>
<td>Neutrophil recruitment and immunity to extracellular pathogens</td>
<td>T₈, T₁₇ cells, CD8⁺ T cells, CD8⁺ T cells, NK cells, NKT cells and LTi cells</td>
</tr>
<tr>
<td>IL-17A-IL-17F</td>
<td>NA</td>
<td>IL-17RA and IL-17RC</td>
<td>Autoimmune pathology (presumed), neutrophil recruitment and immunity to extracellular pathogens</td>
<td></td>
</tr>
<tr>
<td>vIL-17</td>
<td>ORF13</td>
<td>IL-17RA (and IL-17RC)</td>
<td>Unknown</td>
<td>Herpesvirus satellit</td>
</tr>
</tbody>
</table>

CTLA8, cytotoxic T lymphocyte antigen 8; IL-17, interleukin-17 receptor; LTi, lymphoid tissue inducer; NA, not applicable; NKT, natural killer T; ORF, open reading frame; T₈, T₈ helper.
**Effector T Cell Development: Distinct Treg Targets**

<table>
<thead>
<tr>
<th>Non-Lymphoid Tissues</th>
<th>Lymphoid Tissues</th>
<th>Non-Lymphoid Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type 1 Pathogens</strong> (e.g. intracellular bacteria, viruses)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 17 Pathogens (e.g. extracellular bacteria)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **iDC**
  - Migration
  - Macropinocytosis
  - Ag processing

- **mDC**
  - IL-2

- ** iTreg**
  - IL-17
  - IL-23

- **Th1**
  - IFNγ
  - LTα

- **Th17**
  - IL-17
  - IL-17F

- **iTreg**
  - IL-1R
  - IL-23R

- **IL-12R**
  - IL-12
  - IL-23

- **IL-18R**
**Th1-Th2: Host Protection vs Autoimmunity**

**Anti-Pathogen**
- Intracellular Bacteria
- Protozoa
- Viruses
- Tumor eradication

**Anti-Host**
- Rheumatoid Arthritis
- Crohn's disease
- Multiple Sclerosis
- Allograft Rejection
- GVHD

**Pathogenic Role for IL-23, not IL-12, in Autoimmunity**

**Models:** Experimental Autoimmune Encephalomyelitis (EAE) & Collagen-induced Arthritis (CIA)

- **WT**
  - Disease

- **IL-12p35-deficient**
  - Exacerbated disease

- **IL-12p40-deficient**
  - No disease

- **IL-23p19-deficient**
  - No disease

Th1 development: Interplay of Innate and Adaptive Immunity

Type 1 Pathogens (bacteria, viruses, protozoa)

IL-12

IFNγ

STAT1

IL-12Rβ1

IL-12

Antigen, IL-12 + IL-18

Th1

IL-12

IL-18

IFNγ

LTα

MΦ

NK

IFNγ

IL-12

STAT4

IL-12Rβ1/β2

Th1 development: Interplay of Innate and Adaptive Immunity