Innate immune regulation of T-helper (Th) cell homeostasis in the intestine

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## Two arms of immunity

<table>
<thead>
<tr>
<th></th>
<th><strong>Innate immunity</strong></th>
<th><strong>Adaptive immunity</strong></th>
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</thead>
<tbody>
<tr>
<td><strong>Timing</strong></td>
<td>Rapid (hours)</td>
<td>Slow (days)</td>
</tr>
<tr>
<td><strong>Target</strong></td>
<td>Broad</td>
<td>Specific</td>
</tr>
<tr>
<td><strong>Main cells</strong></td>
<td>APCs, PMN, NK, ILCs,</td>
<td>T cells, B cells</td>
</tr>
<tr>
<td><strong>Location</strong></td>
<td>Interface (mucosa, skin)</td>
<td>Internal (lymphoid organs)</td>
</tr>
</tbody>
</table>
Pattern recognition receptors (PRRs) recognize microorganisms.
TLR signaling
Innate immune cells in the intestine (mouse colon)
CD4+ cells in the intestine (normal human colon)
The interplay between innate and adaptive Th cell immunity

Antigen presenting cells encounter microorganisms

DCs

Mφs

Phagocytosed Degraded

Innate Immune recognition

Cytokine
Chemokine
Co-stimulatory molecules

activate

suppress

ET

Reg

Differentiate

naïve

or
Th cell differentiation

- Naïve CD4+ T cells
- TCR
- CD28
- MHC
- APCs
- CD80/86
- IL-12
- IL-4
- IL-10
- TGF-β
- IL-1β
- IL-6
- IL-23
- Th1
- Th2
- Treg
- Th17
- IFN-γ
Distribution difference in Th cell types

Sathaliyawala et al. Immunity 2013
Intestinal commensal bacteria maintain mucosal Th cells especially Th17 type

Macpherson et al., Nature Reviews Immunology 2004

Ivanov et al., Cell 2009
Function of Th17 cells

- Anti-fungal (filamentous fungi, candida, aspergillus)
- Anti-bacterial (especially extracellular bacteria)
- Induce chemotaxis (neutrophils, macrophages)
- Cell growth (in some tumor)
- Induce autoimmune diseases (MS, RA, etc)
- Induce cytokines (IL-17A, IL-17F, IL-22, IL-21, GM-CSF, CCL20, TNF)
- Lineage flexibility (plasticity)
MyD88-dependent TLR4 signaling induces IL-23p19 in APCs

- IL-6
- IL-23
- TGF-β
- IL-1β
- IFN-γ
- IL-12
- TNF-α
- MHC
- TLR4
- CD28
- CD80/86
- CD4+
- T cells
- APCs
- BMDC
- Peritoneal macrophages

JBC 284: 24006–24016, 2009
Does TRIF-dependent TLR4 signaling induce Th17 cell differentiation?

CD4+ T cells

TCR

CD28

MHC

CD80/86

APCs

TLR4

MyD88

TRAM

TRIF

TRAF6

NF-κB

MAPKs

NF-κB

AP-1

IRF3

IL-6

IL-23

TGF-β

IL-1β

IFN-β

TRIF
TRIF-dependent TLR4 signaling likely suppress Th17 cell differentiation

In vitro Th cell differentiation
Co-culture (1:4)

Y. enterocolitica

Salmonella

Infect immun 83: 4404-15, 2015
The role of TRIF signaling in the regulation of Th17 cells in the intestine

In host defense mechanism (Y. enterocolitica infection model)

TRIF-deficient (Trif\textsuperscript{LPS2}) mice generate more Th17 cells and less Th1 cells in response to \textit{Y. enterocolitica} infection (day 9)
TRIF-deficient mice fail to establish effective immunological memory in the intestine

*Y. enterocolitica* re-infection model

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Infect immun 83: 4404-15, 2015
Absence of TRIF skews memory T cells towards Th17 response (7 days post secondary infection)

MLN cells

WT

Trif$^{LPS2}$

CD4+ T cells

WT 27.1±2.2%

Trif$^{LPS2}$ 28.8±7.3%

WT

Trif$^{LPS2}$

CD44

WT

Trif$^{LPS2}$

CD62L

WT 19.7±3.4%

Trif$^{LPS2}$ 14.7±2.5%

WT 22.8±2.2%

Trif$^{LPS2}$ 14.7±2.5%

WT 5.9±0.1%

Trif$^{LPS2}$

T_{CM} T_{EF} T_{CM} T_{EF}
Transferring primed Th cells (role of TRIF in T cells or non T cells?)

- **Trif\textsuperscript{LPS2} mice**
  - 1X10\textsuperscript{5} CFU *Y. enterocolitica*
  - 28 days
  - CFSE label
  - Transfer 6X10\textsuperscript{6}

- **WT mice**
  - 1X10\textsuperscript{5} CFU *Y. enterocolitica*
  - 28 days
  - CFSE label
  - Transfer 6X10\textsuperscript{6}
  - WT CD4+ T cells from the MLN, and the spleen

- **Trif\textsuperscript{LPS2}**
  - High dose infection (5X10\textsuperscript{7} CFU)
Primed T cells do not confer protective immunity without TRIF signaling during bacterial infection

5x10^7 CFU infection after primed T cell transfer

7 days post infection

14 days post infection

Trif\textsuperscript{LPS2} Mfs had an unique cytokine profile that supports Th17 cell differentiation.

52 downregulated in Trif\textsuperscript{LPS2}

35 upregulated in Trif\textsuperscript{LPS2}
Consistent alteration of gene expression in the MLN during *Y. enterocolitica* infection

Infect immun 83: 4404-15, 2015
TRIF-dependent regulation of Th17 cell response involves type I IFN signaling

In vitro Th cell differentiation
Co-culture (1:4)

WT mice

Isolate Peritoneal MØs

WT MØs + T cells + anti-IFNAR

Infect immun 83: 4404-15, 2015
The role of TRIF signaling in the regulation of Th17 cells in the intestine

Summary

① In host defense mechanism (Y. enterocolitica infection model)

1. TRIF suppresses Th17 cell generation and balances Th1 and Th17 cell responses against Gram-negative bacteria, Y. enterocolitica.

2. The maintenance of Th1 and Th17 cell balance also requires TRIF signaling and is involved in host resistance to Y. enterocolitica.

3. TRIF-dependent suppression of Th17 cell generation may be mediated by type I IFN signaling.
The role of TRIF signaling in the regulation of Th17 cells in the intestine

② In intestinal inflammation (TNBS colitis model)

Pathogenesis of inflammatory bowel disease (IBD) involves host Th cell responses to commensal bacteria

In patients with IBD

- Increased number of Th17 cells have been found in inflammed mucosa.
- Attempts have been made to block IL-17, a signature cytokine of Th17 cells in patients with CD; however, it failed to meet efficacy criteria.
- Animal studies have demonstrated inconsistent results with regard to pathogenic and regulatory phenotypes of Th17 cells in the intestine.
- The existence of IFN-γ(+) and Foxp3(+) Th17 cells in human and animal models of IBD suggests the fluctuant phenotype of Th17 cells in the intestine.
- IFN-γ(+) Th17 cells have been implicated in severity of colitis in human and animal models of IBD.
Trif\textsuperscript{LPS2} mice carry IFN-\(\gamma\) expressing Th17 cells in the intestine during TNBS colitis.

IL-27 treatment reduces severity of TNBS colitis in Trif\(^{LPS2}\) mice along with suppression of IFN-\(\gamma\) expressing Th17 cells in the lamina propria.

rIL-27 daily injection (s.c, 0.25\(\mu\)g)

Th17 cell plasticity in Crohn’s disease

Commensal Bacteria

IFN-β

TRIF

IFNAR

STAT1

Naïve

Th17

STAT3

IL-27

IL-10

IL-17(+)

IFN-γ(+) ex-Th17

Plasticity

IFN-γ(-)

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP ID</th>
<th>Alleles</th>
<th>Allele frequency</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>IL27</td>
<td>rs26528</td>
<td>G/A</td>
<td>45.1</td>
<td>9.7*10E-22</td>
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<tr>
<td>IFNAR</td>
<td>rs2284553</td>
<td>G/A</td>
<td>59.9</td>
<td>2.1*10E-16</td>
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<tr>
<td>STAT1</td>
<td>rs1517352</td>
<td>C/A</td>
<td>60.0</td>
<td>1.9*10E-10</td>
</tr>
</tbody>
</table>
The altered expression of cytokine receptor expression in IFN-γ(+) Th17 cells

IFN-γ/IL-17A reporter mouse

- WT
- Isolate Splenic naïve T cells
- Isolate Peritoneal M Cell
- Three days
  - Co-culture (1:4) in the presence of CBA

Microarray analysis

Sorting

IFN-γ

IL-17

WT

Tr

LPS2

f

WT

CBA

IFN-γ/IL-17A reporter mouse
IFN-γ(+)Th17 cells (ex-Th17 cells) are more potent to induce colitis than IFN-γ(-)Th17 cells.
The role of TRIF signaling in the regulation of Th17 cells in the intestine

1. TRIF suppresses Th17 cell generation and plasticity with Th1 cells in response to commensal bacteria (CBA).

2. The suppression of Th17 cell generation and plasticity by TRIF signaling involves IL-27, IFNAR, and STAT1.

3. SNPs of IL-27, IFNAR, and STAT1 were individually associated with human Crohn’s disease.

4. IFN-γ(+)Th17 cells have strong inflammatory capacity especially in the ileum.

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Summary

Mφs

TRIF mediated Type I IFN signaling

Impairs host resistance to infection

Exacerbates colitis

Th1 / Th17 imbalance

Naïve

Plasticity

Th1

Th17

Th17

Th17