Identification of an innate T helper type 17 response to intestinal bacterial pathogens


NATURE MEDICINE

VOLUME 17 | NUMBER 7 | JULY 2011
TGFβ + IL-4

IFN-γ + IL-12
most potent:
NK, CD8, CD4

IL-4 + IL-2
Basophils, Mast cells,
Eosinophils, Nuocytes,
MPPtype2, NHC
Basophil-DCs

TGFβ + IL-6
DCs

TSLPR?

GATA3
pSTAT5

RORγt
pSTAT3

IL-23R

Bcl-6
GATA3

IL-21

IL-4

Tfh
T cell help for B cells

IL-6, IL-21 (autocrine)

IL-12R

IL-12R

IL-18R

T-bet
pSTAT4

GATA3
pSTAT5

RORγt
pSTAT3

TGFβ

FoxP3
Treg
Regulation

Th9
Impact on mast cells

Antiviral and bacterial

Extracellular parasites

Inflammation - fungal immunity
C. rodentium

Th17

Th1

Th2

Th9

IL-9

TGFβ

β3

??

GATA3

PU.1

IL-21

IL-6

IL-4

TGFβ + IL-4

IL-12R

TSLPR?

RORγt
pSTAT3

GATA3
pSTAT5

T-bet
pSTAT4

Naive
CD4
T cell
C. rodentium–induced colitis: robust colonic Th17 response at 2 weeks after infection

Streptomycin-pretreated mice infected with S. typhimurium develop an acute inflammatory response in the cecum, with IL-17 produced early (24-48 h) by \( \gamma \delta \) T cells and other unidentified cells.

Both early (innate) and late (adaptive) IL-17 production in intestinal infections.

What are the innate immune receptors involved in early IL-17 production?

What are the cells producing early IL-17?
Nod1 and Nod2 are involved in early inflammation

*C. rodentium* infection (1 × 10⁹ CFU)

- Nod1⁻/⁻-Nod2⁻/⁻ have less colonic inflammation 7d after *C. rodentium* infection
- Both Nod signaling in radio-resistant and radio-sensitive cells is required for control of infection
Early IL-17 responses are Nod1 and Nod2 dependent

Figure 3: Similar with Salmonella infection (except that Nod signaling is also involved in γδT cell-derived IL-17)
IL-6 induction is required for early Th17 responses

C. rodentium - cecum 4 d after infection
(same for Salmonella)

Nod signal from hematopoietic and non-hematopoietic cells regulate IL-6 induction

Fold change over pre-sort uninfected

cicum increased numbers of CD11c⁺CD11b⁺CD103⁺
Cecum - Salmonella infection at 24 h

Reduction in proportion of CD4+ TCRβ+ expressing IL-17A and IL-22.

Total numbers also drop in anti-IL6 and IL-6-/-.

BM chimeras: IL-6 produced by hematopoietic cells drives IL-17A - IL-22 production

IL-6 induction is required for early Th17 responses
Are innate Th17 conditioned by the microbiota?

The SPF colony is SFB+ but Nod1 and Nod2 does not influence SFB colonization.

Compensatory mechanism?
Also observed following CD4 depletion.
Discussion - Summary

- Nod1-/-Nod2-/- mice do not generate early Th17 responses in the cecum - named iTh17
- Results in delayed pathology and increased disease
- Th17 cells may have innate-like properties
- IL-23 has been shown to regulate innate IL-17 from LT1 and γδT cells but iTh17 require IL-6
- This iTh17 response may not happen in very clean mice?
Foxp3+ follicular regulatory T cells control the germinal center response

Le A Linterman1,2, Wim Pierson3, Sau K Lee2, Axel Kallies4, Shimpei Kawamoto5, Tim F Rayner1, a Srivastava2, Devina P Divekar1, Laura Beaton2, Jennifer J Hogan2, Sidonia Fagarasan5, Adrian Liston3, th G C Smith1,6 & Carola G Vinuesa2,6

Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions

Yeonseok Chung, Shinya Tanaka, Fuliang Chu, Roza I Nurieva, Gustavo J Martinez, Seema Rawal, Yi-Hong Wang, Hoyong Lim, Joseph M Reynolds, Xiao-hui Zhou, Hui-min Fan, Zhong-ming Liu, Sattva S Neelapu & Chen Dong

Affiliations | Contributions | Corresponding authors
TFH cells

- Secreted cytokines are directing CSR and SHM: Reinhardt et al.. Nat Immunol (2009)
- Bcl6 -/-: multiple organs inflammatory disease, elevated IgE, defective GC formation

**What cells control TFH cells?**

- humans: CD4+CD25+CD69- T cells found in GCs
Plasticity of Treg cells

- **Foxp3+ are converted into Tfh in PP:** Tsuji et al. Science (2009)
- IFNγ secretion: Treg cell upregulate T-bet and CXCR3
- High amounts of IRF4, a transcription factor essential for Th2 effector cell differentiation, is dependent on Foxp3 expression. Ablation of a conditional Irf4 allele in Treg cells results in selective dysregulation of Th2 responses, IL4-dependent immunoglobulin isotype production,
- Suppression was lost upon Treg-specific ablation of Stat3, a TF critical for Th17 differentiation, and resulted in the development of a fatal intestinal inflammation.
Retain intense CXCR5 expression - migration to CXCL13-rich areas within GCs

CXCR5+ ICOS+ CD28+ CD40L+ PD-1+ IL21R+ BTLA+,SLAM (CD150)lo, CD122lo CD200hi secrete IL-21

Tfh cell differentiation: ICOSL-dependent (as well as GC formation and antibody production)
TFR are distinct but share similarities with TFH and Treg

SRBC immunization

Similar kinetics as TFH

FoxP3+ cells are present within the Bcl6 area

TFR resemble Treg but also TFH gene expression
Treg: FoxP3, Ctla4, Gitr, Klrg1 and Prdm1, Il10
TFH: Cxcr5, Pdcd1, Bcl6, Cxcl13, Icos
No expression of IL-4 and IL-21 or CD40L
TFR and TFH colocalize: do they require similar signaling cues for their formation?

T-cell priming through CD28 is one of the first signals required for TFH cell development.

**Immunization with SRBC - 7 days after analysis**
TFR and TFH colocalize: do they require similar signaling cues for their formation?

SAP interaction of TFH cell precursors with B cells are required for TFH cell formation/maintenance

Immunization with SRBC - 7 days after analysis

Treg cells formed independently of B cells and were only slightly reduced in the absence of SAP

Development from TFH and TFR are similar and Treg cells differentiate independently of TFH or TFR
TFR co-express Bcl6 and Blimp1

Bcl6 is the transcription factor of TFH and Blimp1 is the transcription repressor. (they mutually repress each other). Is the same true in TFR?
Bcl6 and Blimp1 control TFR formation and homeostasis

i.n influenza infection- 10d after in Mediastinal lymph node

**embryonic 1:1 reconstitution**

- CD45.2 Prdm1gfp/+  
  - CD45.1 Prdm1+/+
- CD45.2 Prdm1gfp/gfp:
  - CD45.1 Prdm1+/+
- CD45.2 Bcl6-/-
  - CD45.1 Bcl6+/+

**unaffected**

**doubles**
Precursors of TFH?

1x10^5 naive CD4+CD25-CD44low TCRHEL
CD45.1+

CD45.2+ B10.BR (I-Ak)

HEL in alum
7d

spleen

all TFR were derived from endogenous T cells

same with OTII-OVA model
Precursors of TFR?

1x10^6 naive CD4+FoxP3-CD44low or CD4+FoxP3+CD44int (CD45.2 FoxP3gfp)

KLH in Ribi

Both donor Treg and naive developed into CCR5+PD-1+ but only Treg cells maintained FoxP3 expression

1x10^6 naive CD4spFoxP3+ thymic Treg or CD4spFoxP3- (FoxP3gfp CD45.2)

CD45.1

SRBC 7 days after

Supplementary fig. 5
### In vivo selective depletion of TFR

8 weeks after reconstitution: DT treatment one day before SRBC immunization and 2 and 5 days after

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Allotype</th>
<th>Tfh*</th>
<th>Treg*</th>
<th>Tfr*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sh2d1a&lt;sup&gt;++&lt;/sup&gt;</td>
<td>CD45.2</td>
<td>50</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>FoxP3&lt;sup&gt;DTR&lt;/sup&gt;</td>
<td>CD45.1</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sh2d1a&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>CD45.2</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>FoxP3&lt;sup&gt;DTR&lt;/sup&gt;</td>
<td>CD45.1</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*These numbers represent the percentage of cells expected to derive from the bone cells in each of the chimeras after immunisation and DT treatment.

---

**TFR suppress TFH cell numbers and B cell numbers**
Do TFR control germinal center B cell selection?

NP-KLH in alum

FoxP3DTR  FoxP3WT

DT 6 days later  analysis 10 days later

No differences in germinal center B cell percentages

NP-sp B cells were reduced in the germinal center

With BM chimeras (Sh2d1a:FoxP3) where TFR are specifically depleted they suggest that antigen-specific B cells are reduced whereas non-antigen specific B cells are increased.
• In response to T-D antigens, Treg cells adopt a TFH differentiation program (Bcl6, CXCR5...)

• They suppress TFH cells and the B cell germinal center response

• Thus: Treg cell can mold their differentiation program to the environment even for TFH cells in the germinal center response.