Effector T cell and polarization

Francesco Annunziato

SIICA School of Immunology, Bari 2017
Index

- Classification of effector Th cell subsets

- Cytokines and transcription factors involved in the differentiation of the three major effector Th cell subsets

- Plasticity of effector Th cell subsets
Naive CD4 T cells have distinct fates that are determined by the pattern of signals they receive from the microbes-activated APC.

Protection to:
- intracellular microbes
- extracellular microbes
- extracellular parasites

Cells of innate compartment
- cytokines

Activity on innate cells:
- IFN-γ
- LT-α
- Macrophages
- NK cells

Pathological condition:
- autoimmune disorders

Protection to:
- extracellular microbes
- intracellular microbes

IL-12

Naive Th
- IL-1β
- IL-6
- IL-23

Th1
- T-bet
- IFNs
- IL-12

Th17
- RORC
- IL-17a
- IL-17f
- IL-22

Th2
- GATA3
- IL-4
- IL-5
- IL-13

Autoimmune disorders:
- Neutrophils
- Macrophages
- Eosinophils
- Mast cells
- Basophils
- allergic disorders

Pathological condition:
- autoimmune disorders
Human effector CD4+ T cells in 2017

1986

- Th2
- GATA3
- IL-4
- IL-5
- IL-9
- IL-13

1986

- IL-2
- IL-4

Naive Th

2005

- IL-1
- IL-23
- TGF-β
- IL-6

Th17
- RORγt
- IL-17A
- IL-17F
- IL-22
- IL-21

IFNs
- IL-12

1986

- Th1
- T-bet
- IFN-γ
- LT-α
Two types of murine helper T cell clones.

I. Definition according to profiles of lymphokine activities and secreted proteins.


<table>
<thead>
<tr>
<th>T Cell</th>
<th>Specificity</th>
<th>Strain</th>
<th>MHC Restriction</th>
<th>IL 2</th>
<th>IL 3</th>
<th>MCGF2</th>
<th>TCGF2</th>
<th>IFN-γ</th>
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</table>

* a Lymphokine activities were evaluated by assays described in the text.
  b CRBC, MHC alleles 2, 14, 15, 19, and 21, but not 13.
  c CRBC, all MHC alleles.
  d CRBC, MHC alleles 2, 13, 14, 15, and 19, but not 21.
  e Not determined.
  f Not applicable.
Human Th1 and Th2 subsets: doubt no more

Parronchi et al., PNAS 88: 4538-4542, 1991
Index

- Classification of effector Th cell subsets

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Th1 polarization; how does it work?

- Cytokines (IFN-α, IFN-γ, IL-12 and IL-18) produced by cells of the innate immunity induce the in vitro development of Th1 cells


<table>
<thead>
<tr>
<th>Cells cultured with:</th>
<th>Proliferative response$^*$</th>
<th>Cell surface phenotype$^+$</th>
<th>Cytokine production$^5$</th>
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<tr>
<td></td>
<td>APC</td>
<td>APC + Der p I</td>
<td>CD4$^+$</td>
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<tr>
<td>Der p I alone</td>
<td>2.1 ± 0.9</td>
<td>43.1 ± 12</td>
<td>1.7 ± 0.5</td>
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<tr>
<td>Der p I + IL-12</td>
<td>3.3 ± 1.6</td>
<td>37.9 ± 7.4</td>
<td>0.9 ± 0.2</td>
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<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.05</td>
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Table 1. Antigen-induced Proliferative Response, Cell Surface Phenotype, and Cytokine Production by Der p I-specific T Cell Lines Obtained in the Absence or Presence of IL-12
IL-12, IL-18 and IFNs in Th1 differentiation

Two pathways can induce the acute transcription of IFN-γ in effector Th1 cells: TCR signalling or the combined triggering of IL-12R and IL-18R.
IL-12 signalling is important for Th1 differentiation. Shi, M., Immunity 28, 763–773 (2008). Naive Th cells Ag DC TCR MHC IL-18Rβ IL-12Rβ1 IL-12Rβ2 IFN-γ STAT1 T-bet STAT4 T-bet T-bet IFN-γ↑ IFN-γ↑↑↑ IFN-γ↑ IFNs I IFN-γ↑↑↑ IFN-γ↑
Evidences coming from in vivo “models”

Mice deficient for STAT1 are impaired in IL-12 production and fail to control Th1 cell-dependent Mycobacterial infection.

Mice deficient in IL-12 p35, p40, IL-12Rβ1 or IL-12Rβ2 display impaired IFN-γ secretion, Th1 differentiation, and NK cytolytic activity.

STAT4-knockout mice are viable, and their phenotype is similar in most respects to mice lacking IL-12 or IL-12R subunits.

Mice deficient for T-bet do not develop Th1 cells and fail to control Th1 cell-dependent protozoan infection.

Mutations of IL-12p40 and IL-12Rβ1 have been reported in patients with recurrent mycobacterial disease.
Th1 immune response

**Th1**

- TCR
- MHC II
- peptide

**B**

- CD40L
- CD40

**NK**

- proliferation
cytolitic activity

**CD8**

- proliferation
cytolitic activity

**Microbicidal activity**

- IL-1
- IL-8
- TNF-α
- chemokines

**Microbicidal activity**

- NO
- IL-1
- IL-6
- IL-8
- TNF-α
- chemokines

**IFN-γ**

- proliferation
cytolitic activity

**IFN-γ**

- TNF-α

**IL-2**

**IL-1**

**IL-6**

**IL-8**

**TNF-α**

**chemokines**

**proliferation**

**cytolitic activity**

**Microbicidal activity**
Th2 polarization: how does it work?
TCR-dependent, exogeneous IL-4-independent pathway

Weak TCR stimulation

Parasites allergens

DC

MHC Ag TCR

Naive Th

GATA-3

IL-4R

IL-4R

STAT5

GATA-3

IL-2

IL-4

Jagged1-Notch

Weak TCR signals

IL-2

IL-4

IL-9

IL-13

Th2

Weak TCR stimulation

IL-2

IL-4R

STAT6

GATA-3

IL-2

IL-4

IL-4R

STAT5

GATA-3

IL-2

IL-4

IL-9

IL-13

Th2
Th2 polarization: how does it work?
TCR-dependent, exogeneous IL-4-dependent pathway

Parasites allergens

DC

MHC Ag TCR

Naive Th

IL-4R

GATA-3

IL-2

STAT5

GATA-3

IL-4

STAT6

GATA-3

IL-2

IL-4

Th2

IL-4

IL-5

IL-9

IL-13

TCR stimulation

? IL-4

how does it work?
T cell independent sources of IL-4

Rag1/Rag2-deficient mice that lack mature B and T cells can still produce substantial amounts of type 2 cytokines.

Type 2 innate lymphoid cells and role for IL-25, IL-33 and TSLP in Th2 cell polarization
The discovery of group 2 innate lymphoid cells (ILC2), which can be involved in the induction of adaptive Th2 responses.
A new player in Th2 polarization: IL-25
A new player in Th2 polarization: IL-33

Cell damage

Epithelium

Fb

DC

IL-33

Mas

IL-25
IL-13

Ba

IL-4
IL-13

DC

MHC

Ag

TCR

Naive
Th

IL-4
STAT6
GATA-3

IL-4R

IL-4R

IL-2

STAT5
STAT6
GATA-3

Th2

IL-13
IL-5
IL-9
IL-13

IL-13
IL-5

ILC2

IL-5
IL-13

IL-25R

IL-33R

IL-33

DC

IL-33R

IL-33R

IL-25

IL-13

IL-4

IL-13

IL-4

IL-13

IL-5

IL-13
Th2 polarization: TSLP

IL-12 ↓↓↓
OX40L ↑↑
Jagged1 ↑↑

Parasites allergens
IL-1β
TNF-α
TLR

epithelium
Parasites
IL-1β
TNF-α
TLR

DC
Naive Th
TSLP
IL-4R
IL-4
STAT6
GATA-3
IL-4
IL-2

Mas
IL-13
IL-5
IL-6

Bas
IL-4

ILC2
IL-13

Th2
IL-4
IL-5
IL-9
IL-13

TSLP
Th2 immune response

Release of toxic proteins and cytokines

Eo → CCL11

IL-5

M

Mucus secretion

En

Ep

IgE

Fb

FcεRI

allergen

Mc/Bas

Release of mediators and cytokines

Th2 → IL-4, IL-9

B → IL-4, IL-9

Mc/Bas → IL-4, IL-9

IL-4, IL-13, IL-9

IL-4, IL-13

IL-4, IL-9

IL-9
**Th17 polarization: how does it work?**

**Combination of cytokines used for Th17 polarization in human**

- TGF-β+IL-1+IL-6+IL-23
- TGF-β+IL-1+IL-23
- TGF-β+IL-21
- IL-1+IL-6
- IL-1+IL-23

### References
- Volpe et al. *(Nat Immunol, 2008)*
- Manel et al. *(Nat. Immunol, 2008)*
- Yang et al. *(Nature, 2008)*
- Acosta et al. *(Nat Immunol, 2007)*
- Wilson et al. *(Nat Immunol, 2007)*
IL-1 and IL-23, but not IL-6, IL-21 or TGF-β induce Th17 associated molecules in naive UCB CD4+ cells.
IL-1 and IL-23 induce both IL-17 and IFN-γ in UCB CD4+ cells
CD161 and CCR6 are markers of IL-17-producing cells


Cosmi et al., J Exp Med 2008
CD161 identify all circulating IL-17-producing T cells

Maggi et al., Eur J Immunol. 2010
UCB CD4+CD161+ T cells constitutively express RORC, IL-23R and CCR6 and can be induced to differentiate towards IL-17-producing cells.
CD161 identify all UCB IL-17-cell precursors

Anti-CD3 plus anti-CD28 mAbs in the presence of IL-1β plus IL-23
1 wk

Maggi et al., Eur J Immunol. 2010
Is TGF-β required for differentiation of CD4+CD161+ naïve T cells into Th17?

10% FCS medium  serum free medium

Santarlasci et al., Eur J Immunol 2009
Is TGF-β required for differentiation of CD4+CD161+ naïve T cells into Th17?

Santarlasci et al., Eur J Immunol 2009
Th17 cells are less susceptible than Th1 and Th2 cells to the TGF-β suppressive activity
Human Th17 cells exhibit reduced expression of Clusterin when compared to Th1 cells.

Santarlasci et al., Eur J Immunol 2009
Clusterin, a novel modulator of TGF-β signaling, is involved in Smad2/3 stability

Kwan-Bok Lee a, Jun-Ho Jeon b, Inpyo Choi c, O-Yu Kwon d, Kweon Yu e, Kwan-Hee You a,*

Biochemical and Biophysical Research Communications 366 (2008) 905-909
Human Th17 cells exhibit lower TGF-β-induced apoptosis and increased Bcl-2 expression compared to Th1 cells.

Santarlasci et al., Eur J Immunol 2009
Human Th17 development: our point of view

Annunziato et al., Int. Immunol, 2008
Findings in mouse!

**IL-1 is critical for murine Th17 differentiation, which may occur even in the absence of exogenous TGF-β**
*Chung et al., Immunity 30, 576, 2009*

**Transforming growth factor-β is dispensable for the molecular orchestration of Th17 cell differentiation**
*Das et al. J. Exp. Med. online September 28, 2009*

**Generation of pathogenic Th17 cells in absence of TGF-β signaling.**
*Ghoreschi, et al. Nature 467; 967, 2010*
Loss of function of TGF-βRII or complete loss of TGF-βRI in CD4+ cells does not impair Th17 development but increases Th1 cell numbers in vivo

Mouse Naïve CD4+ cells can be polarized in vitro into Th17 cells by IL-1β, IL-6 and IL-23 cytokines.

Generation of pathogenic Th17 cells in absence of TGF-β signaling.

Th17 cell network

Non immune cells

Th17

CXCL8

CCL20

Endothelium

Epithelium (skin, lung, intestine)

IL-17A
IL-17F
IL-22
IL-26

IL-17A
IL-17F

CKX8

IL-21

NK cells
CD8 T cells

B cells

Macrophages

Neutrophils

Fibroblast

Immune cells

Annunziato et al., Trends in Immunology in press 2012
Index

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CD4+CD161+ T cells are increased in inflamed tissues and include high number of Th1 cells

*Crohn disease* (gut)

*Psoriasis* (skin)

*Juvenile idiopathic arthritis* (SF)

Cosmi et al., *Arthritis Rheum*. 2011
CD4+CD161+IFN-γ+ (non-classic Th1), but not CD4+CD161-IFN-γ+ (classic Th1), cells express Th17 associated markers.

Cosmi et al., Arthritis Rheum. 2011
Principal component analysis of genes expressed by Th17, non-classic and classic Th1 clones.
Ability of Th17 cells to shift towards Th1 phenotype

CD4+CD161+CCR6+ derived T cell clones consist of Th1, Th2, Th17, Th17/1 and Th17/2 cells

CD161+ T cell clones

CD161- T cell clones

red dots: IFN-γ producing cells

T-bet cDNA (fg/10000 cells)

GATA-3 cDNA (fg/10000 cells)

RORC cDNA (fg/10000 cells)

Cosmi et al., JACI 2010
Th17/2 cell clones produce both type 2 and type 17 cytokines

Cosmi et al., JACI 2010
CD161+CCR6+ Th17/Th2 cells are able to induce IgE production and are significantly higher expressed in PB from asthmatic patients.
CD4+CD161+ UCB precursors cultured in the presence of IL-1, IL-23 and IL-4 don’t differentiate towards Th17/Th2 cells

Cosmi et al., JACI 2010
Repeated stimulation of CD4+CD161+CCR6+ cells in the presence of IL-4 induce Th17 cells to shift into IL-4 producing clones

Cosmi et al., JACI 2010
Human Th17 cells plasticity update

Published October 4, 2010

JEM
A novel subset of CD4⁺ T_H2 memory/effector cells that produce inflammatory IL-17 cytokine and promote the exacerbation of chronic allergic asthma

Yui-Hsi Wang, Kui Shin Voo, Bo Liu, Chun-Yu Chen, Burcin Uygungil, William Spoede, Jonathan A. Bernstein, David P. Huston, and Yong-Jun Liu

IL-4

CD161

RORC

GATA-3

CCR6

IL-12

CD161

RORC

T-bet

IL-23R

IL-12Rβ2

CCR6

Th17

Th2

IL-4

CD161

RORC

T-bet

IL-12

CD161

RORC

T-bet

CD161

RORC

T-bet

Th17

Th1
CRTH2 is a selective marker of human Th2 and Tc2 cells

CD4+CRTH2+ T cells can be polarized into IFN-γ-producing cells by IL-12.

**Annunziato et al. JACI 108:815, 2001**
Interferons Direct Th2 Cell Reprogramming to Generate a Stable GATA-3^+T-bet^+ Cell Subset with Combined Th2 and Th1 Cell Functions

Ahmed N. Hegazy,1,2 Michael Peine,1,2,6 Caroline Helmstetter,1,2,6 Isabel Panse,1,2 Anja Fröhlich,1,2 Andreas Bergthaler,3 Lukas Flatz,4 Daniel D. Pinschewer,5 Andreas Radbruch,2 and Max Löhning1,2,*
CD4 T cell plasticity

**Classic Th1**

- T-bet
- IFN-γ
- LT-α

**IFNs**

- IL-12

**Naive Th**

- IL-1β
- IL-23

- IL-4
- IL-25
- IL-33
- TSLP

**Th17**

- IL-17a
- IL-17f
- IL-22

**Th2**

- IL-4
- IL-5
- IL-13
- GATA3

**Non-classic Th1**

- IFN-γ

**IL-12**

Th17/Th1

- RORC

Th17/Th2

- RORC
- GATA3

Th0

- GATA3
- T-bet