Regulation of Th17 and Treg cells in the mucosal surfaces

Daniel Mucida
La Jolla Institute for Allergy and Immunology
Advances on T helper cells research

- First evidence suggesting that T cells helping antibody production and mediating DTH are different
- IL-4 induces switch to IgE
- Th1 protects, Th2 exacerbates Leishmaniasis
- CD25+ cells are important for maintenance of self-tolerance
- GATA3 is required for Th2 development
- T-bet controls Th1 lineage commitment
- TGF-beta plus IL-6 are the differentiation factors for Th17
- Th17 is proposed as a new T helper lineage
- RORgammat is the transcription factor for Th17 cells
- TGF-beta plus IL-21 can also drive Th17

Basso AS and Mucida D; Cell Res. 2009
Th1/Th2 paradigm
Inconsistencies in the Th1/Th2 paradigm to explain autoimmune diseases

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Haplotype</th>
<th>Incidence</th>
<th>Mean day of onset</th>
<th>Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>GKO−/−</td>
<td>d</td>
<td>17/24</td>
<td>19.6 ± 2.1</td>
<td>4 ± 0.6</td>
</tr>
<tr>
<td>GKO+/−</td>
<td>d</td>
<td>4/47</td>
<td>23.8 ± 4.8</td>
<td>3.25 ± 1.3</td>
</tr>
<tr>
<td>GKO++/++</td>
<td>d</td>
<td>1/16</td>
<td>26</td>
<td>1</td>
</tr>
</tbody>
</table>

Krakowski, M EJI 1996

Bettelli, E JEM 2004

Segal BM, JEM 1998
Discovery of IL-23 (p19-p40)
IL-23 rather than IL-12 is required for EAE*

*Cua, D Nature 2003

Similar findings in other autoimmune diseases models
IL-23 is required for induction of IL-17 producing CD4 cells (Th17 cells)

Harrington, L Nat Immunol 2005

Park, H Nat Immunol 2005
TGF-β and IL-6 are required for initial development of Th17 cells from naïve CD4 T cells

Bettelli, E  Nature 2006
Mangan, PR Nature 2006
Veldhoen, M Immunity 2006
**RORγt** is the transcription factor involved in Th17 development.
Sequential development of Th17 cells

Bettelli E.; Nature 2008
IL-23 rather than IL-12 is required for the development of inflammatory and autoimmune diseases initially linked to a Th1-type of response (EAE, arthritis, IBD);

IL-23 is required for the development of IL-17 producing CD4 cells;

IL-17 producing CD4 T cells (Th17 cell) constitute an unique pathway of T helper development, different from- and inhibited by- Th1 or Th2-related cytokines;

TGF-β and IL-6 (and/or IL-21) are required for initial development of Th17 cells while IL-23 is required for the stabilization of the Th17 phenotype;

Th17 development requires the transcription factor RORγt (and STAT3).

\[ EAE = \text{experimental autoimmune encephalomyelitis; IBD = inflammatory bowel disease} \]
Current T helper-cell paradigm

- iTₖₑğ_reg (FOXP3)
  - TGF-β
- iTₖₙₐïve
  - IL-4
  - TGF-β + IL-6
- Tₖₜ₁
  - T-bet
  - Clearance of intracellular pathogens
  - Immunopathology
  - Autoimmunity
- Tₖₜ₂
  - GATA3
  - Clearance of extracellular pathogens
  - Allergy
  - Atopy
- Tₖₜ₁₇
  - ROR-γ
  - Clearance of certain classes of extracellular pathogens (bacteria (for example, Klebsiella spp.) and fungi)
  - Tissue inflammation
  - Immunopathology
  - Autoimmunity

Bettelli E.; Nature 2008
## Effector functions of Th17 cells

<table>
<thead>
<tr>
<th>Disease model</th>
<th>IL-17</th>
<th>IL-17F</th>
<th>IL-21</th>
<th>IL-22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental autoimmune encephalomyelitis</td>
<td>Pathogenic(^{13,15})</td>
<td>ND</td>
<td>Pathogenic(^{60,61})</td>
<td>None(^{57})</td>
</tr>
<tr>
<td>Collagen-induced arthritis</td>
<td>Pathogenic(^{31,32})</td>
<td>ND</td>
<td>Pathogenic(^{31,5})</td>
<td>ND</td>
</tr>
<tr>
<td>Acute colitis</td>
<td>Protective(^{28})</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Chronic colitis</td>
<td>Pathogenic(^{290})</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Asthma</td>
<td>Pathogenic (priming phase)? Protective (effector phase)(^{36})</td>
<td>ND</td>
<td>Protective(^{117})</td>
<td>ND</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>None</td>
<td>ND</td>
<td>ND</td>
<td>Protective(^{58})</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>ND</td>
<td>ND</td>
<td>Pathogenic(^{118})</td>
<td>ND</td>
</tr>
</tbody>
</table>

IL, interleukin; ND, not determined; T\(_H\), T helper.

Dong C.; *Nat Rev Immunol* 2008
Dual role of IL-17 in asthma

Tanaka, S JI 2009
Dual role of IL-17 in asthma: required for optimal sensitization ...
Dual role of IL-17 in asthma: attenuates allergic responses

Schnyder-Candrian, S JEM 2006
He R, PNAS 2007
IL-17 in pulmonary responses

Nembrini C.; J Allergy Clin Immunol 2009
Mucida D.; J Allergy Clin Immunol 2009
Th17 and Treg cells require TGF-β

Th1 (T-bet)

IFNγ, IL-12

Naive CD4 T cell

IL-4

IL-6, TGFβ

Th2 (GATA-3)

Th17 (RORγt)

TGFβ, RA, IL-2

Tfh (Bcl-6)

iTreg (Foxp3)
Mechanisms of TGF-β dependent immune functions

TGF-β is highly produced in the intestine and is crucial for the induction of both, peripheral regulatory T cells and inflammatory Th17

High frequency of Treg cells
Mucida et al. Science 2007

High frequency of Th17 cells
Mucida et al. unpublished
The intestinal mucosa is the largest area of exposure to environmental antigens

(Around 300 m²)
The intestinal mucosae is chronically exposed to large amounts of pathogenic and non-pathogenic *non-self* antigens.

**Non commensal**
- Major site for invasive bacteria and parasites infection

**Commensal**
- $10^{12}$ microorganisms per g of feces

**Diet**
- 80g of proteins/day
GALT (Gut Associated Lymphoid Tissue)

More T and B cells than all other compartments together
Mucosal Immunity
Balance between protective immunity and immune tolerance
1- Oral tolerance

“Inhibition of immune responsiveness to subsequent challenge of proteins previously ingested”

• 1829 - R. Dakin - inflammatory reaction after ingestion of poison ivy

• 1909 - A. Besredka – oral administration of milk prevented anaphylaxis to milk injection

• 1911 - H.G. Wells - feeding vegetable proteins prevented anaphylaxis to them

• 1946 - M.W. Chase - feeding DNCB to guinea pigs prevented DTH

• 1975 - 1983 - Thomas & Parrot, Andre, Vaz, Titus & Chiller, Saklayen - more systematic studies on the phenomenon of “oral tolerance”

Resuscitation of oral tolerance: Vaz and coworkers


<table>
<thead>
<tr>
<th>FED</th>
<th>CHALLENGE</th>
<th>RESPONSE (10^3 PFC/SPL)</th>
<th>% SUPPRESSION</th>
</tr>
</thead>
<tbody>
<tr>
<td>SALINE</td>
<td>OVA</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>OVA</td>
<td>OVA</td>
<td>28</td>
<td>97</td>
</tr>
<tr>
<td>SALINE</td>
<td>HGG</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>OVA</td>
<td>HGG</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>
Which cells are responsible for oral tolerance?
Oral tolerance induction in mice lacking natural regulatory T cells

TCR: OVA-specific  BCR: HA-specific

**Oral Exposure to OVA**
Day -7 to -3

**Immunization with OVA/HA**
Day 0
i.p. Injection with OVA/HA -Alum
14 days

**Intranasal Challenge**
with OVA/HA
Days 14 and 21

**Sac. Day 22**
BAL: Cells, Abs and cytokines
Serum, mLN, spleen
Oral feeding suppresses an asthma-type inflammatory response in mice that lack thymus-derived regulatory T cells (nTreg).

**BAL cells**
- Cells x 10^-5
- None, Imm, Tol
- Histogram with bars for Macrophages, Lymphocytes, Neutrophils, Eosinophils

**Serum antibodies**
- IgE
- Anti-HA IgG1
- None, Imm, Tol
- Graphs showing concentration in µg/ml and titer x 10^-3

Oral exposure to TCR-specific protein readily induces peripheral Treg cells while suppress effector T cell development.

Oral exposure to proteins induces tolerance and adaptive Foxp3⁺ Treg cells in a TGFβ-dependent manner
Oral tolerance induces Foxp3+ peripherally induced Treg cells in a TGFβ-dependent manner.
Which cells mediate Treg induction at the intestinal surfaces?
Intestinal DCs specifically express RALDH and produce retinoic acid (RA)
Mucosal DCs promote Foxp3 expression via retinoic acid (RA) production
Regulation of Th17 differentiation by a population of mucosal DCs

Mucida et al. Science 2007
Mucosal DC suppress Th17-differentiation via retinoic acid (RA) production

\begin{align*}
\text{IFN-\(\gamma\)} & \quad \text{IL-17} \\
\text{Splenic DC} & \quad \text{Intestinal DC}
\end{align*}

\begin{align*}
\text{none} & \quad TGF-\beta+IL-6 \\
0.3 & \quad 8.4 \\
4.9 & \quad 1
\end{align*}

Mucida et al. Science 2007
Mucida et al. \textit{unpublished data}
RA counteracts IL-6 in IL-17 x Foxp3 induction

**Naïve B6 CD4 cells + anti-CD3 + SPL APCs (4 days in culture)**

Mucida et al. Science 2007
Protection of colitis by TGF-β/RA treated cells

Naïve CD4 T cells $\rightarrow$ Rag1-/- mouse $\rightarrow$ Analyses

In vitro cultured T cells $\frac{1}{2}$ ratio

$CD45RB^{high} + \text{none}$

$CD45RB^{high} + \text{TGF-β/RA}$

Histological Score

Days post-transfer

Mucida et al. Science 2007
Balance between inflammatory and suppressive responses is tightly regulated in the intestine
Development and regulation of Th17 cells in the intestine

Mucida D.; J Allergy Clin Immunol 2009
IL-17 producing cells in the intestine depend on the stimulation by commensal microbiota (Segmented Filamentous Bacteria)- Ivanov at al. *Cell* 2009;

ATP derived from the commensal bacteria is involved in the production of IL-17 by lamina propria CD4 T cells through induction of IL-6, IL-23 and TGF-β production by intestinal DCs;

Retinoic acid, also produced by intestinal DCs, induces gut homing of T cells primed in the mesenteric lymph nodes;

Retinoic acid acts as a co-factor inducing regulatory T cells and efficiently suppress Th17-cell development;

Retinoic acid is required for optimal Th2 responses but is able to suppress chronic allergic responses.
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