IL-2 paradoxically controls tolerance and immunity to established tumors \textit{in vivo}

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Interleukin-2

“Although it has been 25 years since the identification and initial characterization of IL-2, its precise function in the physiology of the immune system remains enigmatic.”

The IL-2 Paradox

1) Historically IL-2 was called **T cell growth factor** for its ability to grow T cells *in vitro*

2) In contrast, mice which are deficient in IL-2 or its signaling components have lymphoproliferative and multi-organ autoimmune disease

3) Therefore, it appears that the dominant function of IL-2 *in vivo* is the maintenance of self-tolerance

4) It is now accepted that IL-2 maintains T$_{reg}$ cell homeostasis *in vivo*
1) A distinct lineage of CD4$^+$ T cells, which **constitutively** express CD25, CTLA-4, GITR, and Foxp3

2) Express **Foxp3**, a transcription factor, which is related to T$_{\text{reg}}$ function.

3) Need IL-2 for expansion **in vitro** and **in vivo**. Are dysfunctional but present in IL-2$^{-/-}$ and IL-2R$\alpha^{-/-}$ mice, but are completely absent in Foxp3 deficient mice.

T$_{\text{Reg}}$ - CD4$^+$CD25$^+$Foxp3$^+$

T$_{\text{H}}$ - CD4$^+$CD25$^{lo}$Foxp3$^{-}$
The Idea

CD4^+CD25^+ T_R

CD4^+CD25^- T_H → Autoimmune Disease

RAG-1 KO
The Experimental Model

*Tumor bearing RAG-1 KO*

PF $T_H (+T_R)$ → No Tumor Immunity

PF $T_H$ → Tumor Immunity

P - pmel-1 CD8$^+$ T cells (recognize gp100)
F - Fowlpox hgp100 vaccine
The Experimental Model

A

CD45.1 \( T_R \)  

CD45.2 \( T_H \)

B

-7 to -10 d  
1 wk  
2 wk  
3 wk  
4 wk

Tumor Inoculation

Treatment

Analyze
Treg cells inhibit and T helper cells augment effective adoptive immunotherapy and autoimmunity

Antony et. al. March 2005, Journal of Immunology
CD4 Response
T\textsubscript{reg} cells and IL-2R signaling control the size of CD4\textsuperscript{+} T cell compartment

\begin{figure}
\centering
\includegraphics[width=\textwidth]{chart.png}
\caption{T\textsubscript{reg} cells and IL-2R signaling control the size of CD4\textsuperscript{+} T cell compartment.}
\end{figure}

$\text{T}_{\text{reg}}$ cells and IL-2R signaling control the size of CD4$^{+}$ T cell compartment.
$T_{\text{reg}}$ cells require IL-2 from $T_{\text{helper}}$ cells for maintenance of the high affinity IL-2R and Foxp3 expression.

**A**

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Week 3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treg</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Treg Alone</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Treg/Th WT</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Treg/Th IL-2/-</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Treg/Th CD25/-</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

* P<0.001

**B**

<table>
<thead>
<tr>
<th></th>
<th>Treg Alone</th>
<th>Treg (+Th)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foxp3 Expression</td>
<td>0</td>
<td>600</td>
</tr>
</tbody>
</table>
**T**<sub>reg</sub> **cells require IL-2 from T**<sub>helper</sub> **cells for maintenance of the high affinity IL-2R**
IL-2 controls the frequency of the T\textsubscript{reg} cell population

A

<table>
<thead>
<tr>
<th>Foxp3</th>
<th>CD25</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>IL-2 KO</td>
</tr>
</tbody>
</table>

*FL2-H: CD25 PE*  
*FL1-H: FOXP3 FITC*  

*10^0, 10^1, 10^2, 10^3, 10^4*
IL-2 signaling is required for the competitive fitness of Treg cells in the periphery
IL-2 signaling is not essential for T helper cell function but is critical for T\(_{\text{reg}}\) cell function/homeostasis.

A  
CD\(_4^+\)CD\(_{25}^-\)\(-\) T\(_H\) cells

Pretransfer  
3-4 wks

B

![Flow cytometry graph](image)

- No treatment
- PF
- PF Th CD25 KO (+Treg)
- PF Th CD25 KO

Days Post Treatment
Summary of the CD4 response \textit{in vivo}

1) IL-2 from Th cells regulates CD25 expression on Treg cells in the periphery and controls Foxp3 expression

2) IL-2 signaling is coupled to $T_{\text{reg}}$ cell homeostasis and survival/expansion ("fitness") \textit{in vivo} and possibly may also be required for their suppressive mechanism

3) T helper cells do not need high affinity IL-2R to help
CD8 Response
“There is currently no evidence of a role for IL-2 …” with regard towards helping CD8\(^+\) T cell responses \textit{in vivo}.

**CD8+ T cells need IL-2 to initiate anti-tumor immunity and maintain their numbers**

(a) Tumor Area (mm²)

- No treatment
- PF
- PF Th
- PF Th IL-2 KO
- PF Th CD25 KO

Days Post Treatment

(b) No help

T_reg

T_h WT

T_h (+T_reg)

T_h CD25 KO

T_h IL-2 KO

(c) P > 0.05 NS

P = 0.01

P < 0.05

Number of pmel-1 T cells (x 10⁷)

No help

T_reg

Th

Thc/T_reg

Th IL-2 KO

Th CD25 KO

Vb13
IL-2R signaling is required for CD8$^+$ T cell function *in vivo*

**Graph:**
- **Legend:**
  - No Treatment
  - P(CD25 KO) V
  - P V

- **X-axis:** Days Post Treatment
- **Y-axis:**
  - 0 to 400

**Data Points:**
- **P-** pmel-1 naïve T cells (1e6)
- **V-** Vaccinia Virus hgp100
$T_{\text{reg}}$ cells suppress generation of the effector response \textit{in vivo}
Summary: CD8\(^+\) T cells and IL-2 *in vivo*

1) CD8\(^+\) T cells need help in the form of IL-2 for effective immunity to self in the absence of Treg cells

2) However, in the presence of Treg cells, IL-2 preferentially activates Treg cells

3) To emphasize this, CD8\(^{CD25KO}\) T cells, which cannot respond to IL-2, do not treat tumors
Conclusions

1) IL-2 signaling appears to be more critical for $T_{\text{reg}}$ cells and CD8$^+$ T cells than for CD4$^+$ T helper cells *in vivo*

2) Therefore, exogenous IL-2 therapy may be preferentially expanding $T_{\text{reg}}$ cells *in vivo*

3) Therapies that block the activation of Treg cells and enhance T effectors cells will be more beneficial for immunotherapy
Anti-IL-2 plus IL-15 augments adoptive immunotherapy in lymphodepleted mice
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