Timing Matters in T Cell Differentiation

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  - Deepa Sathaye, Alexander Moser

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Outline

System

Antigen presenting cell (APC)

Naïve T cell

Regulatory T (Treg) cells

Helper T (Th) cells

Methodology

Model design

Model elements

Influence sets (Interaction map)

Set of discrete values for each element

Influence table

Model rules

Model simulations

Circuit design methods

Experiments

Expert knowledge

Literature

Model analysis
T cell subpopulation ratios are critical for numerous immune and auto-immune pathologies.

Determinants of the peripheral T cell differentiation are not completely understood.
Questions to address

- How can we predict the antigen dose that will induce Treg versus Th?
- Are there signaling cascades in T cells that are critical in this cell fate decision?
- Can we use modeling to identify the critical factors?
- Many clinical trials involving DC vaccines do not take antigen dose into account
- Could also be important for the ex vivo expansion of Treg for therapeutic purposes
Modeling goals

- Determine whether known mechanisms are sufficient to explain experimental observations
- Suggest additional experiments to identify missing mechanisms and clarify areas of uncertainty
- Identify early markers of the response
Network model

**Receptors:**
- T cell receptor (TCR)
- Co-stimulation through CD28
- IL-2 receptor (IL-2R)
- TGFβ receptor (TGFβ R)

**Transcription factors:**
- AP-1, NFAT, NFκB, SMAD3, STAT5

**Genes:**
- IL-2, CD25, Foxp3

**Other important elements:**
- PTEN, PI3K, PIP3, PDK1,
- Akt, mTORC1, mTORC2, TSC1-TSC2, Rheb, S6K1, pS6
Five scenarios

1. High antigen dose
2. Low antigen dose
3. High antigen dose, then removed
4. High antigen dose and TGFβ
5. High antigen dose, then inhibitors added
Scenarios 1 and 2: High and Low antigen dose
Scenarios 1 and 2: High and Low antigen dose

Source: N. Miskov-Zivanov et al., in preparation.
Scenarios 1 and 2: High and Low antigen dose

Source: N. Miskov-Zivanov et al., in preparation.
Scenarios 1 and 2: High and Low antigen dose

Experiments

Simulations

Source: N. Miskov-Zivanov et al., in preparation.
Scenario 1: High antigen dose

**Value:**
- ON (1)
- OFF (0)

**Network Diagram:**
- APC
- MHC
- CD86
- TCR
- CD28
- PI3K
- PIP3
- PDK1
- SMAD3
- PTEN
- S6K1
- mTORC1
- mTORC2
- TSC1-TSC2
- AKT
- STAT5
- NFAT
- NFκB
- Fos
- Jun
- AP-1
- NFαB
- Foxp3
- FOXP3
- IL-2Rα
- IL-2
- IL-2R
- JAK3
- RAF
- TAK1
- PKC-θ
- MEK2
- MKK7
- ERK
- JNK
- Ras
- Ca²⁺
Scenario 1: High antigen dose
Scenario 1: High antigen dose

- APC
  - MHC
  - CD86

- TCR
  - CD28

- TGFβ
  - TGFβR
  - IL-2R

- PI3K
  - PIP3
  - PDK1
  - AKT
  - PDK1

- SMAD3
  - STAT5

- mTORC1
  - S6K1
  - RHEB

- NF-κB
  - AP-1
  - FOXP3

- NFAT
  - IL-2Rα
  - IL-2

- PTEN

- JAK3

- Raf
  - TAK1

- Ca2+

- MEK2
  - MKK7

- ERK
  - JNK

- Ras
  - PKC-θ

- TSC1-TSC2

- γ δ

- α

Value = ON (1)
Value = OFF (0)
Scenario 1: High antigen dose
Scenario 1: High antigen dose
Scenario 1: High antigen dose
Scenario 1: High antigen dose
Scenario 1: High antigen dose trajectory

Trajectory example

<table>
<thead>
<tr>
<th>TCR_HIGH</th>
<th>PI3K_HIGH</th>
<th>PTEN</th>
<th>PI3K</th>
<th>AKT</th>
<th>MTORC1</th>
<th>S6K1</th>
<th>MTORC2</th>
<th>STAT5</th>
<th>IL-2</th>
<th>CD25</th>
<th>FOXP3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>value = ON (1)</td>
<td>value = OFF (0)</td>
<td>value = ON (1)</td>
<td>value = OFF (0)</td>
<td>value = ON (1)</td>
<td>value = OFF (0)</td>
<td>value = ON (1)</td>
<td>value = OFF (0)</td>
<td>value = ON (1)</td>
<td>value = OFF (0)</td>
<td>value = ON (1)</td>
<td>value = OFF (0)</td>
</tr>
</tbody>
</table>
Scenario 1: High antigen dose trajectory

Trajectory example

TCR_HIGH
PI3K_HIGH
PTEN
AKT
MTORC1
S6K1
MTORC2
STAT5
IL-2
CD25
FOXP3

value = ON (1)
value = OFF (0)
Scenario 2: Low antigen dose trajectory

Trajectory example
Scenario 2: Low antigen dose trajectory

Trajectory example

<table>
<thead>
<tr>
<th>TCR_LOW</th>
<th>PI3K_LOW</th>
<th>PTEN</th>
<th>PIP3</th>
<th>AKT</th>
<th>MTORC1</th>
<th>S6K1</th>
<th>MTORC2</th>
<th>STAT5</th>
<th>IL-2</th>
<th>CD25</th>
<th>CD28</th>
</tr>
</thead>
<tbody>
<tr>
<td>ON (1)</td>
<td>OFF (0)</td>
<td>ON</td>
<td>ON</td>
<td>ON</td>
<td>OFF</td>
<td>OFF</td>
<td>ON</td>
<td>ON</td>
<td>ON</td>
<td>ON</td>
<td>OFF</td>
</tr>
</tbody>
</table>

Node values:
- value = ON (1)
- value = OFF (0)
Scenarios 1 and 2: High and Low antigen dose

Experiments

Simulations

Source: N. Miskov-Zivanov et al., in preparation.
Model does not capture low dose scenario

- Model simulations of low antigen dose result in 100% Foxp3+ cells, in experiments no more than 50% Foxp3+ cells
Model does not capture low dose scenario

- Model simulations of low antigen dose result in 100% Foxp3+ cells, in experiments no more than 50% Foxp3+ cells

Low dose antigen is modeled as a change in rules:

- \[ \text{PKCTHETA}^* = \text{TCR\_HIGH} \lor (\text{TCR\_LOW} \land \text{CD28} \land \text{MTORC2}) \]
- \[ \text{PI3K\_LOW}^* = (\text{TCR\_LOW} \land \text{CD28}) \lor (\text{PI3K\_LOW} \land \text{IL2\_EX} \land \text{IL2R}) \]
- \[ \text{PI3K\_HIGH}^* = (\text{TCR\_HIGH} \land \text{CD28}) \lor (\text{PI3K\_HIGH} \land \text{IL2\_EX} \land \text{IL2R}) \]
- \[ \text{PTEN}^* = (\neg \text{TCR\_HIGH} \land \text{PTEN}) \lor (\neg \text{TCR\_HIGH} \land \text{FOXP3}) \]
Model does not capture low dose scenario

- Model simulations of low antigen dose result in 100% Foxp3+ cells, in experiments no more than 50% Foxp3+ cells

Low dose antigen is modeled as a change in rules:

- **PKCTHETA\(^*\) = TCR\_HIGH or (TCR\_LOW and CD28 and MTORC2)**
- **PI3K\_LOW\(^*\) = (TCR\_LOW and CD28) or (PI3K\_LOW and IL2\_EX and IL2R)**
- **PI3K\_HIGH\(^*\) = (TCR\_HIGH and CD28) or (PI3K\_HIGH and IL2\_EX and IL2R)**
- **PTEN\(^*\) = (not TCR\_HIGH and PTEN) or (not TCR\_HIGH and FOXP3)**

A more dynamic analysis of TCR signal strength necessary: duration of stimulation
Analysis of duration of stimulation

Experiments

Scenario 3: Antigen removal at rounds 1-12 (T1-T12)

<table>
<thead>
<tr>
<th>Attractors</th>
<th>T6</th>
<th>No removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3</td>
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<tr>
<td>A4</td>
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<td>A5</td>
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<td>A6</td>
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<td>A7</td>
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<td>A8</td>
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<td>A9</td>
<td></td>
<td></td>
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<tr>
<td>A10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foxp3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ras</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI3K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Akt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mTORC1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mTORC2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Attractor size:
- 40, 6, 17, 3, 374, 13, 127, 1, 118, 126, 175, 1000, 1000
Scenario 3:
Antigen removal at rounds 1-12 (T1-T12)

1000 simulation trajectories

Average trajectories for attractor 1010111001
Scenario 3: Antigen removal at round 6 (T6)

Experiments

Model simulations
Scenario 3: Antigen removal at round 6 (T6)

Experiments

Model simulations

Experiments

Model simulations
Time to reach steady state (T6, HD, LD)

- Treg cells take longer to differentiate than Th cells.
### Model checking

- **SPIN provides yes/no answers**

<table>
<thead>
<tr>
<th>Specification</th>
<th>LTL Formula</th>
<th>Verified</th>
<th>Specification</th>
<th>LTL Formula</th>
<th>Verified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Does Foxp3 become false forever?</td>
<td>!(&lt;&gt;[]!foxp3)</td>
<td>Yes</td>
<td>Does Foxp3 become true forever?</td>
<td>!(&lt;&gt;[]foxp3)</td>
<td>Yes</td>
</tr>
<tr>
<td>Does ps6 become true forever?</td>
<td>!(&lt;&gt;[]ps6)</td>
<td>Yes</td>
<td>Does pS6 stay false forever?</td>
<td>!([]!ps6)</td>
<td>Yes</td>
</tr>
<tr>
<td>Does PIP3 become true forever?</td>
<td>!(&lt;&gt;[]pip3)</td>
<td>Yes</td>
<td>Does IL-2 become true but eventually become false forever?</td>
<td>!(&lt;&gt;il2 &amp;&amp; &lt;&gt;[]!il2)</td>
<td>Yes</td>
</tr>
<tr>
<td>Does Akt become true forever?</td>
<td>!(&lt;&gt;[]akt)</td>
<td>Yes</td>
<td>Is PTEN always true?</td>
<td>!([]pten)</td>
<td>Yes</td>
</tr>
<tr>
<td>Does mtTORC1 become true forever?</td>
<td>!(&lt;&gt;[]mtorc1)</td>
<td>Yes</td>
<td>Does mTORC become true forever?</td>
<td>!(&lt;&gt;[]mtorc)</td>
<td>Yes</td>
</tr>
<tr>
<td>Does S6K1 become true forever?</td>
<td>!(&lt;&gt;[]s6k1)</td>
<td>Yes</td>
<td>Does CD25 become true forever?</td>
<td>!(&lt;&gt;[]cd25)</td>
<td>Yes</td>
</tr>
<tr>
<td>Does STAT5 become true forever?</td>
<td>!(&lt;&gt;[]stat5)</td>
<td>Yes</td>
<td>Does STAT5 become true forever?</td>
<td>!(&lt;&gt;[]stat5)</td>
<td>Yes</td>
</tr>
<tr>
<td>Does CD25 become true forever?</td>
<td>!(&lt;&gt;[]cd25)</td>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Does IL-2 become true forever?</td>
<td>!(&lt;&gt;[]il2)</td>
<td>Yes</td>
<td>Does IL-2 become true forever?</td>
<td>!(&lt;&gt;[]il2)</td>
<td>Yes</td>
</tr>
<tr>
<td>Does PTEN become true forever?</td>
<td>!(&lt;&gt;[]pten)</td>
<td>Yes</td>
<td>Does PTEN become false forever?</td>
<td>!(&lt;&gt;[]!pten)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**High dose + TGFβ**

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Probabilistic model checking

- PRISM allows for analyzing transient behavior

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Does Foxp3 eventually become false forever?</td>
<td>$\Pr=?[\text{FG}\neg\text{foxp3}]$</td>
<td>1</td>
<td>Does pS6 stay true?</td>
<td>$\Pr=?[\text{G}\text{ps6}]$</td>
<td>0</td>
</tr>
<tr>
<td>What is the probability that Foxp3 never becomes true?</td>
<td>$\Pr=?[\text{G}\neg\text{foxp3}]$</td>
<td>0.765098</td>
<td>Does mTORC2 ever get to true?</td>
<td>$\Pr=?[\text{F}\text{mtorc2}]$</td>
<td>1</td>
</tr>
<tr>
<td>Does pS6 become true forever?</td>
<td>$\Pr=?[\text{F}\text{ps6}]$</td>
<td>1</td>
<td>Does PTEN ever get to false?</td>
<td>$\Pr=?[\text{F}\neg\text{pten}]$</td>
<td>0</td>
</tr>
<tr>
<td>Are IL-2 and PTEN ever both true?</td>
<td>$\Pr=[\text{F}(\neg\text{pten}\land\neg\text{il2})]$</td>
<td>0.000468</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>What is the probability that IL-2 is false before Foxp3 becomes true</td>
<td>$\Pr=?[\neg\text{il2}\lor\text{foxp3}]$</td>
<td>0</td>
<td>Does IL-2 become true?</td>
<td>$\Pr=?[\text{F}\text{il2}]$</td>
<td>0.019584</td>
</tr>
</tbody>
</table>

**PRISM – behavior over time**

<table>
<thead>
<tr>
<th>Specification</th>
<th>LTL Formula</th>
<th>Figure</th>
<th>Specification</th>
<th>LTL Formula</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>What is the behavior of Foxp3 over time?</td>
<td>$\Pr=?[\text{G}[t,t]\text{foxp3}]$</td>
<td>Figure</td>
<td>What is the behavior of IL-2 over time?</td>
<td>$\Pr=?[\text{G}[t,t]\text{il2}]$</td>
<td>Figure</td>
</tr>
</tbody>
</table>

Deepa Sathaye, Alexander Moser
Probabilistic model checking

- PRISM allows for analyzing transient behavior
Statistical model checking

Statistical model checker

- Model Simulations
- Trace statistics
- More traces?
- New model
- New experiments
Low antigen dose scenario:

1. **Does IL-2 always go to 1?**
   Property: $F[20] (IL2 == 1)$
   Test: BEST 0.001 0.999
   Result: estimated probability close to 1

2. **Probability that IL-2 stays at 0 before Foxp3 becomes 1?**
   Property: $(IL2 == 0) U[15] (FOXP3 == 1)$
   Test: BEST 0.0001 0.999
   Result: estimated probability = 0.00147 rare event

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**Statistical model checking**

- High antigen dose + antigen removal scenario:
  - Studies of relative timing on mTOR vs. CD25/STAT5 pathway

<table>
<thead>
<tr>
<th></th>
<th>Property</th>
<th>Probability estimate and sample size</th>
<th>Elapsed time [s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$G^7 \sim (\text{MTORC1} = 1 &amp; \text{MTORC2} = 1)$</td>
<td>estimate = 0.0188048 samples = 200,160</td>
<td>1,946</td>
</tr>
<tr>
<td>2</td>
<td>$F^7 (\text{MTORC1} = 1 &amp; \text{MTORC2} = 1)$</td>
<td>estimate = 0.980884 samples = 2,352</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>$F^{10} (\text{MTORC1} = 1 &amp; \text{MTORC2} = 1 &amp; \text{CD25} = 0 &amp; (F^{18} (\text{CD25} = 1)))$</td>
<td>estimate = 0.60104 samples = 25,968</td>
<td>253</td>
</tr>
<tr>
<td>4</td>
<td>$F^{28} (\text{MTORC1} == 1 &amp; \text{MTORC2} == 1 &amp; \text{CD25} == 0 &amp; (F^1 (\text{CD25} == 1)))$</td>
<td>estimate = 0.592195 samples = 26,160</td>
<td>254</td>
</tr>
<tr>
<td>5</td>
<td>$F^{10} (\text{MTORC1} = 1 &amp; \text{MTORC2} = 1 &amp; \text{CD25} = 0 &amp; (F^1 (G^{17} (\text{CD25} = 1))))$</td>
<td>estimate = 0.396669 samples = 25,920</td>
<td>254</td>
</tr>
</tbody>
</table>

---

Antigen removal

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Conclusion

- Logical modeling approach allows development of comprehensive models of cell fate
  - Model of peripheral T cell differentiation recapitulates a wide range of experimental observations
  - Circuit analysis reveals key elements of the mechanism for Foxp3 expression
  - Timing is critical for Treg differentiation:
    - Treg cells take longer to differentiate than Th cells
    - Race between Foxp3 activating and inhibiting pathways
    - Feedback between Foxp3 and PTEN
Conclusion

- **Model checking**
  - Allows for more efficient studies of the model
  - Probabilistic model checking:
    - Provides transient results that match simulations
  - Statistical model checking:
    - Further analysis of transient behavior
    - Provides insights into timing relationships between elements
Next steps

- Analyze different removal scenarios using model checking
- Expansion of the model (keep up with fast pace of developments in the field)
- Develop a model for several cell types
- Develop population models that embed intracellular circuitry
Thank you!