Contents

- Rheumatoid arthritis (RA)
- Treatments
- Model overview
- Disease induction
- Scoring
- Typical results - prophylactic treatment
- Typical results - therapeutic treatment
- Positive controls
- Short-term alternative models for first-pass screen
  - LPS stimulation in vivo (C57BL/6 mice)
  - LPS stimulation ex vivo (Lewis rats)
Rheumatoid arthritis (RA)

- Autoimmune disease affecting joints
  - Also connective tissue in heart, blood vessels, lungs, eyes, skin, kidneys
- Affects more women than men (~2:1)
- Joint inflammation is characterized by pain, swelling and stiffness
  - Development of adhesions
  - Erosion of joint surfaces and bone resorption
  - Loss of function and joint deformation

Rheumatoid arthritis treatments

- NSAIDs
- Corticosteroids
- Methotrexate
- TNF-blocking
  - (Enbrel, Humira, Remicade, Simponi, Cimzia)
- IL-1 blocking (Kineret, Ilaris)
- IL-6 blocking (Actemra)
- IL-12/IL-23p40 blocking (Stelara)
- B-cell depleting (Rituxan)
- CTLA4-Ig (Orencia)
CIA in DBA/1 mice – Model overview

- Disease is induced by immunization with chicken or bovine type II collagen emulsified in Complete Freund’s adjuvant (CFA)
- Arthritis develops 17-25 days later
- Prophylactic studies
  - Treatment from immunization, for 6 weeks
- Therapeutic studies
  - Treatment from disease onset, for 14-20 days
- Excellent predictive value for RA

CIA induction and development

Day 0: Immunization with type II collagen/CFA
Day 18: Booster with type II collagen/IFA
Arthritis 4-10 days later
Prophylactic treatment – starts at immunization (Day 0)
Therapeutic treatment starts at arthritis onset
CIA Scoring

- Animal score is total of all 4 paw scores on scale of 0-16
- Each paw is scored from 0 to 4
- Score 0 – Normal paw
- Score 1 – One toe inflamed and swollen
- Score 2 – More than one toe, but not entire paw inflamed and swollen, or mild swelling of entire paw
- Score 3 – Entire paw inflamed and swollen
- Score 4 – Very inflamed and swollen or ankylosed paw

Score 0

[Images of paws with and without inflammation]
**Score 1**

- Swollen toe
- Starting to swell

**Score 2**

- All toes swollen
- No ankle swelling
Score 3

Swollen toes
Swollen ankle

Score 4

Ankylosed paw
Prophylactic treatment

- Treatment from immunization, for 6 weeks
- Mice assigned to groups in a *balanced* manner to achieve similar weight at the time of immunization

![Graph showing mean CIA score + SEM over time for different treatments.](image)
Prophylactic treatment with dexamethasone and methotrexate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CIA incidence (%)</th>
<th>p value</th>
<th>Median day of onset (all mice)</th>
<th>p value</th>
<th>Mean day of onset (sick mice) (+/- SEM)</th>
<th>p value</th>
<th>Maximum CIA score (+/- SD)</th>
<th>p value</th>
<th>End CIA score (+/- SD)</th>
<th>p value</th>
<th>End (%) body weight (+/- SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>100.0%</td>
<td></td>
<td>22.0</td>
<td></td>
<td>26.4 +/- 15.6</td>
<td></td>
<td>11.1 +/- 2.0</td>
<td></td>
<td>11.1 +/- 2.0</td>
<td></td>
<td>87.4 +/- 5.5</td>
<td></td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>0.0%</td>
<td>&lt;0.001</td>
<td>n/a</td>
<td>n/a</td>
<td>9.0 +/- 0.0</td>
<td>&lt;0.001</td>
<td>6.0 +/- 0.0</td>
<td>&lt;0.001</td>
<td>6.0 +/- 0.0</td>
<td>&lt;0.001</td>
<td>94.9 +/- 4.8</td>
<td>0.2011</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>86.7%</td>
<td>0.0877</td>
<td>26.0</td>
<td>0.8886</td>
<td>26.9 +/- 1.2</td>
<td>0.3016</td>
<td>6.7 +/- 4.2</td>
<td>0.3515</td>
<td>6.7 +/- 4.2</td>
<td>0.3515</td>
<td>96.1 +/- 0.5</td>
<td>0.0126</td>
</tr>
</tbody>
</table>

p<0.05
p<0.10
n/a, not applicable, median day of onset cannot be calculated because less than 50% of mice developed disease

Therapeutic treatment

- Treatment from disease onset, for 14-20 days
  - Balanced assignment of mice to groups
    - Similar distribution of day of CIA onset
    - Similar distribution of initial CIA scores
    - Remove likely outliers (late onset)
  - Mice are assigned to groups one at a time, as each shows initial signs of CIA
  - Very consistent groups yield very tight results
Therapeutic treatment – Balanced distribution of mice

<table>
<thead>
<tr>
<th>Group Treatment</th>
<th>Day of Onset +/- SD</th>
<th>CIA, onset score +/- SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>27.0 +/- 1.4</td>
<td>2.47 +/- 1.19</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>26.9 +/- 1.0</td>
<td>2.73 +/- 1.83</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>27.1 +/- 1.0</td>
<td>2.40 +/- 0.83</td>
</tr>
</tbody>
</table>

Therapeutic treatment with dexamethasone and methotrexate

Dose of methotrexate reduced due to toxicity

* p<0.05
Methotrexate is only marginally efficacious in therapeutic treatment of DBA/1 mice
Therapeutic treatment with anti-TNF and CTLA-4-Ig

Effects of anti-TNF and CTLA-4-Ig in therapeutic treatment of CIA

<table>
<thead>
<tr>
<th>Group treatment</th>
<th>Day of CIA onset ± SD</th>
<th>Score at onset ± SD</th>
<th>Maximum score ± SD</th>
<th>p value</th>
<th>End score ± SD</th>
<th>p value</th>
<th>End body weight (% of onset) ± SD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>25.5 ± 1.6</td>
<td>2.3 ± 1.2</td>
<td>13.0 ± 3.0</td>
<td>0.7206</td>
<td>13.0 ± 3.0</td>
<td>0.7206</td>
<td>93.8 ± 4.2</td>
<td>0.5146</td>
</tr>
<tr>
<td>mlgG2a</td>
<td>25.4 ± 1.4</td>
<td>2.3 ± 1.3</td>
<td>12.9 ± 3.8</td>
<td></td>
<td>12.9 ± 3.8</td>
<td></td>
<td>95.1 ± 5.5</td>
<td></td>
</tr>
<tr>
<td>Anti-TNF</td>
<td>25.7 ± 1.1</td>
<td>2.2 ± 1.1</td>
<td>6.2 ± 4.3</td>
<td>0.0012</td>
<td>5.3 ± 4.0</td>
<td>0.0006</td>
<td>194.0 ± 3.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>CTLA4-Ig</td>
<td>25.7 ± 1.1</td>
<td>2.3 ± 1.1</td>
<td>7.4 ± 2.5</td>
<td>0.0025</td>
<td>7.1 ± 2.9</td>
<td>0.0022</td>
<td>101.1 ± 4.1</td>
<td>0.0062</td>
</tr>
</tbody>
</table>

*p<0.05
Therapeutic treatment with anti-TNF and CTLA4-Ig – Histological analysis

<table>
<thead>
<tr>
<th>mlgG2a</th>
<th>anti-TNF</th>
<th>CTLA4-Ig</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Histological Image 1" /></td>
<td><img src="image2.png" alt="Histological Image 2" /></td>
<td><img src="image3.png" alt="Histological Image 3" /></td>
</tr>
<tr>
<td><img src="image4.png" alt="Histological Image 4" /></td>
<td><img src="image5.png" alt="Histological Image 5" /></td>
<td><img src="image6.png" alt="Histological Image 6" /></td>
</tr>
</tbody>
</table>

Histological analysis

* p <0.05

CIA in DBA/1 Mice NON-CONFIDENTIAL © 2011-2014 Hooke Laboratories, Inc.
**Short-term PK/PD models**

- First-pass screens for RA drugs
- LPS stimulation in vivo
  - C57BL/6 mice
- Whole-blood ex-vivo LPS stimulation
  - Lewis rats

**LPS stimulation in vivo (C57BL/6 mice)**

- Compound treatment
- 1 hour
- LPS injection into C57BL/6 mice
- 2 hours
- Blood collection
- Plasma isolation (PK)
- Serum isolation
- Cytokine measurement
LPS stimulation in vivo (C57BL/6 mice)

* p<0.05

Whole-blood ex-vivo LPS stimulation (Lewis rats)

- Plasma isolation for PK
- Whole blood stimulation with LPS (or other stimuli)
- 2-5 hours incubation
- Plasma isolation
- Cytokine measurements
Whole-blood ex-vivo LPS stimulation (Lewis rats)

![Graph showing TNF in serum (pg/mL)]

* * p<0.05

Conclusion

- CIA in DBA/1 mice is an excellent model of rheumatoid arthritis (RA)
- Excellent correlation between efficacy in CIA and RA
  - Positive controls are all approved treatments
- Short term models can be run as preliminary tests of efficacy in CIA
Thank you!

Hooke Laboratories, Inc.
439 South Union Street
Lawrence, MA 01843
USA

Telephone:  +1 617 475 5114
Fax:  +1 617 395 1352
Email:  info@hookelabs.com

http://hookelabs.com