CIA Induction in DBA/1 Mice

Recommended protocol for use with:

- Hooke Kit™ Chicken Collagen/CFA Emulsion (EK-0210) and
- Hooke Kit™ Chicken Collagen/IFA Emulsion (EK-0211)

or

- Hooke Kit™ Bovine Collagen/CFA Emulsion (EK-0220) and
- Hooke Kit™ Bovine Collagen/IFA Emulsion (EK-0221)

Summary

DBA/1 mice develop collagen induced arthritis (CIA) after immunization with collagen emulsified in Complete Freund’s Adjuvant (CFA), followed by a booster dose of collagen emulsified in Incomplete Freund's Adjuvant (IFA).

Maximum arthritic score will be 10 to 14 (on the scale of 0-16) for most mice.

When using chicken collagen, CIA will develop 16-35 days after immunization in 90–100% of male and female mice.

When using bovine collagen, CIA will develop 16-35 days after immunization in 90–100% of male and 60% to 100% of female mice.

Typically, mice are observed for 40 to 60 days.

Materials needed

<table>
<thead>
<tr>
<th>Qty</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hooke Kit™ containing collagen/CFA emulsion (Chicken – EK-0210 or Bovine – EK-0220)</td>
</tr>
<tr>
<td>1</td>
<td>Hooke Kit™ containing collagen/IFA emulsion (Chicken – EK-0211 or Bovine – EK-0221)</td>
</tr>
<tr>
<td>20</td>
<td>DBA/1 mice, males or females 8 to 12 weeks old (Taconic Farms model DBA1BO)</td>
</tr>
</tbody>
</table>
Mice

Either male or female DBA/1 mice may be used.

All mice should be 8 to 12 weeks old at immunization.

Mice must be acclimated to your facility for at least 7 days before immunization.

Male mice must be obtained at not more than 6 weeks of age and therefore are acclimated longer before immunization. This will prevent fighting among cage-mates.

Mature males may be moved with new cage-mates after CIA onset. This is often done when assigning mice to therapeutic groups. Mature males with CIA will not fight one another.

Method

To induce CIA in DBA/1 mice with chicken type II collagen, use cat. no. EK-0210 and EK-0211.

To induce CIA in DBA/1 mice with bovine type II collagen, use cat. no. EK-0220 and EK-0221.

Daily dosing of mice with a potential therapeutic often delays the onset of CIA and decreases incidence and severity of CIA due to stress of the procedures. Careful handling of the mice will minimize these effects. If significant stress is expected, collagen/CFA instead of collagen/IFA may be used for the booster dose to obtain more severe CIA.

The following procedures are identical for both chicken and bovine type II collagen.
Initial immunization with collagen/CFA emulsion – Day 0

1. Immobilize the mouse using a restrainer.

2. Clean the tail with 70% ethanol, wipe the area dry with sterile gauze.

3. Position the syringe containing collagen/CFA emulsion parallel with the tail, pointing the tip of the needle toward the body of the mouse, over the space between the ventral and lateral vein of the tail.

4. Puncture the skin approximately 25 mm (1 inch) distal of the hair line. Insert needle subcutaneously 7 to 10 mm toward the body of the mouse. (This injection is often called intradermal, because there is very limited subcutaneous space under the skin of tail.) Make sure that the needle is visible under the skin. It is important that the only puncture in the skin is at the place where the needle first entered the subcutaneous space.

5. Press firmly at the site of needle entry to prevent any back-leakage of emulsion during the injection. Inject 0.05 mL of the emulsion. You will see the white emulsion entering the subcutaneous space, spreading all the way to the hairline if injected properly. Keep the needle inserted for 10 to 15 seconds after the injection, to avoid leakage of the emulsion. Alternatively, a light pull on the syringe plunger will prevent the leakage. Release the mouse back to the cage.

6. Repeat the procedure with all the mice.

Note – Two to three weeks after immunization, some female mice may develop excessive inflammation at the base of the tail; this is rare in male mice. These mice will undergo significant hair loss and the area surrounding the anus will appear red, inflamed and sometimes moist. Remove these mice from the study as they will not develop CIA optimally for accurate results.
Booster dose with collagen/IFA emulsion – Day 18 to 21

The booster dose should be administered 18 to 21 days after immunization.

If the study requires removal of mice that develop CIA before receiving the booster dose, we recommend administering the booster dose on Day 18 instead of the more commonly used Day 21; this will retain the 10-20% of mice that typically develop CIA by Day 21 in the study. CIA severity will be similar whether the booster dose is administered on Day 18 or Day 21.

1. Immobilize the mouse using a restrainer.
2. Clean the tail with 70% ethanol, wipe the area dry with sterile gauze.

    Note – Blood vessels in the tail will be dilated as a result of the initial immunization.

3. Position the syringe/needle containing type II collagen/IFA emulsion parallel with the tail, pointing the tip of the needle toward the body of the mouse. The emulsion will be injected between the ventral and lateral vein, on the same side of the tail that received the initial injection. The booster dose should be injected several millimeters cranial of the site of the initial immunization (closer to the head of the mouse).

4. Insert the needle 7 to 10 mm into the subcutaneous space. Make sure the needle is visible under the skin and clear of any obvious blood vessels. It is important that the only puncture in the skin is at the place where the needle first entered the subcutaneous space. Be careful to position the needle away from dilated blood vessels and inject very slowly. Accidental injection of emulsion into blood vessels will lead to an embolism and result in death of the mouse.

5. Press the needle and the tail very tightly with your fingers, pressing at the site of needle entry to prevent any back-leakage of emulsion during the injection (see picture).

6. Very slowly (over 30 to 45 seconds), inject 0.05 mL of the emulsion. Keep the needle inserted into the subcutaneous space for another 10 to 15 seconds after the injection, to avoid leakage of the emulsion. Alternatively, a light pull on the syringe plunger will prevent the leakage. Release the mouse back to the cage.

7. Repeat the procedure with all the mice.

    Note – Because blood vessels in the tail are dilated as a result of the immunization, slow delivery of the emulsion is critical to avoid mouse deaths resulting from embolisms. An embolism is indicated by the mouse gasping or rolling in its cage after receiving the booster dose. Such mice should be euthanized, as an embolism usually results in death within thirty minutes. If the mouse does not die immediately, it will develop lung inflammation and will
likely die within 4 to 10 days. Mice which survive embolisms often will not develop CIA optimally.

Check for initial signs of CIA – Starting on Day 14

Check all mice for signs of CIA daily (see CIA scoring), starting on Day 14 after the immunization. (Once enrolled into treatment groups, mice may be scored every other day.)

As soon as the first signs of joint inflammation occur, provide mice with food pellets and wet food on the floor of the cage, and easily accessible water. HydroGel (ClearH2O, Portland ME) may be used as a source of water.

CIA Scoring

We recommend scoring CIA on the scale of 0 to 16 (0 to 4 for each paw, adding the scores for all 4 paws), using the following criteria:

<table>
<thead>
<tr>
<th>Paw Score</th>
<th>Clinical Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal paw.</td>
</tr>
<tr>
<td>1</td>
<td>One toe inflamed and swollen.</td>
</tr>
<tr>
<td>2</td>
<td>More than one toe, but not entire paw, inflamed and swollen, OR</td>
</tr>
<tr>
<td></td>
<td>Mild swelling of entire paw.</td>
</tr>
<tr>
<td>3</td>
<td>Entire paw inflamed and swollen.</td>
</tr>
<tr>
<td>4</td>
<td>Very inflamed and swollen paw or ankylosed paw. If the paw is ankylosed, the mouse cannot grip the wire top of the cage.</td>
</tr>
</tbody>
</table>

Expected Results

CIA is consistently induced 16-35 days after immunization in 90–100% of male mice, in 90-100% of female mice using chicken collagen, and in 60–100% of female mice using bovine collagen.

Maximum severity for most mice will be 10 to 14 (on the scale of 0 to 16).

Approximately 10-20% of mice will show signs of CIA before Day 21 after immunization.
The following illustrates typical results:

### CIA induction in DBA/1 mice

<table>
<thead>
<tr>
<th>Exp #</th>
<th>Mice/group</th>
<th>Age at immunization</th>
<th>Mean maximum score ± SD</th>
<th>Day of onset ± SD</th>
<th>Disease incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>7 weeks</td>
<td>12.71 ± 3.98</td>
<td>26.3 ± 6.2</td>
<td>100 %</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>8 weeks</td>
<td>12.63 ± 3.24</td>
<td>24.4 ± 3.9</td>
<td>100 %</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>8 weeks</td>
<td>13.71 ± 3.72</td>
<td>23.7 ± 5.6</td>
<td>100 %</td>
</tr>
</tbody>
</table>

Data are from three independent experiments using Hooke Kits™ Chicken Collagen/CFA Emulsion (EK-0210) and Chicken Collagen/IFA Emulsion (EK-0211), with female DBA/1 mice (Taconic Farms).

Similar results are obtained using Bovine Collagen/CFA Emulsion (EK-0220) and Bovine Collagen/IFA Emulsion (EK-0221) in male DBA/mice.

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