



Hooke Laboratories

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).

Mean maximum arthritic score will be 10 to 14 (on the scale of 0-16).

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

Qty	Description
1	Hooke Kit™ containing collagen/CFA emulsion (Chicken - EK-0210 or Bovine - EK-0220)
1	Hooke Kit™ containing collagen/IFA emulsion (Chicken - EK-0211 or Bovine - EK-0221)
20	DBA/1 mice, males or females 8 to 10 weeks old (Taconic Farms model DBA1BO)

Method

To induce CIA in DBA/1 mice with chicken type II collagen, use Cat # EK-0210 and EK-0211.

To induce CIA in DBA/1 mice with bovine type II collagen, use Cat # EK-0220 and EK-0221.

The following procedures are identical for both bovine and chicken type II collagen.

Mice should be 8-10 weeks old, acclimated at your facility for at least 7 days before immunization.

1. Immunize mice with collagen/CFA emulsion:

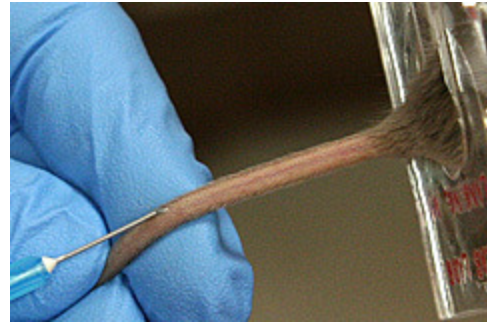
Immobilize the mouse using a restrainer.

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

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 dorsal and lateral vein of the tail.

Insert the needle 7 to 10 mm into the subcutaneous space. (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.

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. 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.

Repeat the procedure with all the mice.

Note – Two to three weeks after the initial immunization, the caudal end of some mice may develop excessive inflammation. 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.

2. Administer booster dose of collagen/IFA emulsion 3 weeks after the initial immunization:

Immobilize the mouse using a restrainer.

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.

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 dorsal 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). Choose an injection location that is a bit paler than other areas – this indicates blood vessels that are less dilated.

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.

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).

Very slowly (over 10 to 15 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.

Repeat the procedure with all the mice.

Note – Because blood vessels in the tail are dilated as a result of the initial immunization, slow delivery of the emulsion is critical to avoid mouse deaths resulting from formation of 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 normally develop lung inflammation and die within four to seven days. If the mouse survives, it will not develop CIA optimally.

3. Check mice for signs of CIA every 2 to 3 days (see CIA scoring), starting on day 14 after the immunization.

Repeat the procedure with all the mice.

4. 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. Transgel (Charles River Laboratories) 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:

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

Expected Results

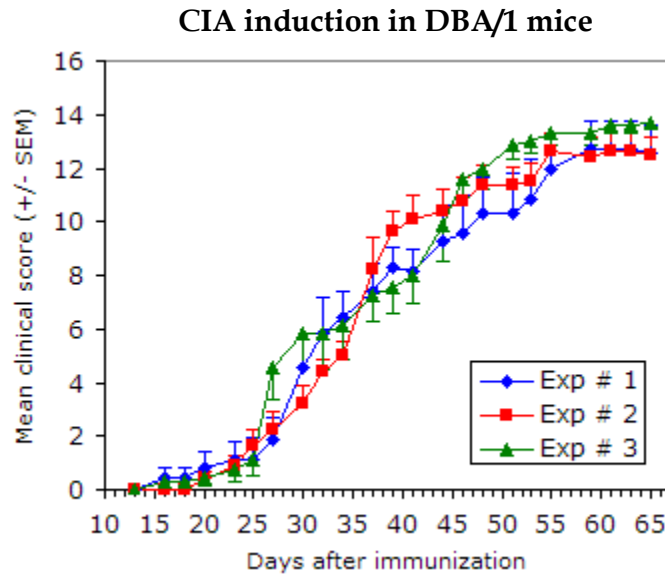
CIA is consistently induced 16-35 days after immunization in 90–100% of male mice, in 90-100% of female mice using chicken collagen, and in 60–100% of female mice using bovine collagen.

Mean maximum severity will be 10 to 14 (on the scale of 0 to 16).

Up to 30% of mice will show signs of CIA even before they receive the booster dose of collagen/IFA on day 21 after the initial immunization. The severity of CIA in these mice will increase after they receive the booster dose.

Daily dosing of mice with a potential therapeutic often delays the onset of CIA and decreases severity of CIA due to stress of the procedures. Careful handling of the mice will minimize these effects. To reduce effects of stress, we recommend sham dosing on several days before immunization of the mice.

The following illustrates typical results:



Exp #	Mice/group	Age at immunization	Mean maximum score \pm SD	Day of onset \pm SD	Disease incidence
1	7	7 weeks	12.71 \pm 3.98	26.3 \pm 6.2	100 %
2	8	8 weeks	12.63 \pm 3.24	24.4 \pm 3.9	100 %
3	7	8 weeks	13.71 \pm 3.72	23.7 \pm 5.6	100 %

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

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