Protocol for the Successful Induction of Collagen-Induced Arthritis (CIA) in Rats

Collagen-induced arthritis (CIA) was originally established in Wistar (outbred), Sprague-Dawley (outbred) and Wistar-Lewis (inbred) rats immunized with type II collagen (1), and later expanded to mice (2) and non-human primates (3). These CIA models have been widely used as a model of human rheumatoid arthritis (RA), since they share both immunological and pathological features with RA. However, there are several differences among species with respect to the CIA model. Susceptibility to CIA is linked to the MHC types. For example rat CIA is less restricted by the MHC type (5), but mouse CIA is highly restricted by the MHC type. Thus, a variety of rat strains with different MHC types develop arthritis, although the incidence and severity of arthritis, as well as antibody and T-cell epitope specificity varies among individual strains (6).

Although the rat CIA model is highly reproducible, certain considerations must be taken into account to successfully induce arthritis with sufficient incidence and severity. The following are considered to be the most important factors to successfully induce rat CIA. These factors must be studied beforehand, especially for first time users of this model.

1. Animal Vendors

From vendor to vendor and even within the same strain, the genetic background and bacteria flora will vary among rats. These differences affect how the rats will respond to various reagents, thus impacting the results of your experiments (7, 16). Chondrex recommends testing animals from different vendors using a defined protocol before proceeding with a full-scale experiment.

2. Housing Condition & Diet

Chondrex recommends housing animals in Specific Pathogen Free (SPF) conditions rather than conventional conditions to avoid variations within experiments caused by bacterial and viral infections. For example, Lewis rats are highly responsive to CIA and the incidence and severity of arthritis is very high. However, the susceptibility to CIA in this strain varies notably depending upon the age, the breeder, and the inoculated intestinal flora (Terato, et al., unpublished observation).

It is well known that diet affects the incidence and severity of CIA in mice (8). However, this has not been extensively studied in rats. Diet may not be as important for rats since they respond to type II collagen quicker and develop arthritis within a shorter period of time compared to mice. However, benefit may be gained by also feeding a high fat diet as is recommended for mice.

3. Rat Age & Strains

Rats should be at least 7-8 weeks old with a mature immune system.

The following strains of rats are known to be highly susceptible to CIA: Sprague-Dawley (SD)(RT1f), Wistar (RT1u)(1), BioBreeding/ Diabetes-resistant (BB/DR)(RT1u) (9), Wistar Furth (RT1u), Louvain (LOU) (RT1u), Osborne-Mendal (OM) (RT1u), and Lewis (LEW) (RT1l) rats (5) (Table 1).

BB rats are the best strain to use for CIA (Biomedical Research Models, Inc., Massachusetts, USA). Dark Agouti (DA) rats are highly susceptible to CIA, and less susceptible to oil-induced arthritis (mineral oil: IFA, alone) (11). Therefore CIA in DA rats may simultaneously involve two different pathogenic mechanisms. Thus DA rats are not recommended for the CIA model.

Note 1: Rat susceptibility to CIA is linked to the MHC RT1 locus and varies with the species of type II collagen used for immunization. In general, porcine type II collagen is the most arthritogenic species of type II collagen followed by bovine, with chicken being the least arthritogenic (5).

Note 2: In contrast to mice, rats immunized with autologous rat type II collagen will develop arthritis (1).

Note 3: Rat antibodies to type II collagen are highly specific to the conformation of the collagen molecule. Denatured collagen and individual CB peptide fragments of type II collagen are not arthritogenic in rats which is contrary to mice (13) and monkeys (12).

Table 1 - Rat strains used for CIA

Strains	MHC RT1 Haplotype	References	Notes
Sprague-Dawley (SD)	f	1	
Wistar	u	1	
Lewis (LEW)	l	1,5	MDP can be effective in LEW rats for higher incidence and severity of CIA (10).
BioBreeding (BB)/ Diabetes-resistant (DR)	u	9	
Wistar Furth	u	5	
Louvain (LOU)	u	5	
Osborne-Mendal (OM)	u	5	
Dark Agouti (DA)	av1	5	Susceptible to oil-induced arthritis

2607 151st Place NE
Redmond, WA 98052, USA

Phone: (425) 702-6365 or (888) 246-6373
Fax: (425) 882-3094

www.chondrex.com
support@chondrex.com

Adjuvant

Since rats are generally susceptible to adjuvant-induced arthritis (AIA), incomplete Freund's adjuvant (IFA), catalog # 7002, must be used.

Catalog #	Description
7002	Incomplete Freund's Adjuvant, 5 ml

Collagen

Highly purified type II collagen prepared under a defined protocol should be used since deglycosylation of collagen will affect the arthritogenicity (14). Moreover, the failure to remove minor contaminants such as pepsin likely yields false positive reactions in a T-cell stimulation assay. Lyophilized collagen is very stable if properly stored at -20°C in the dark. Collagen should be dissolved at 2-4 mg/ml in 0.05M acetic acid by gently stirring overnight at 4°C. Collagen solutions can be kept at 4°C for one week, but should then be kept at -20°C thereafter. Chondrex offers a complete line of immunization grade type II collagen:

Catalog #	Description
20011	Chick type II collagen, 10 mg
20012	Chick type II collagen, 5 ml x 2 mg/ml
20021	Bovine type II collagen, 10 mg
20022	Bovine type II collagen, 5 ml x 2 mg/ml
20031	Porcine type II collagen, 10 mg
20032	Porcine type II collagen, 5 ml x 2 mg/ml
20041	Rat type II collagen, 5 mg
20042	Rat type II collagen, 2.5 ml x 2 mg/ml

Protocols to Induce Arthritis

A. Preparation of Emulsion

The quality of the emulsion for immunization is critical for inducing arthritis with high incidence. Emulsions can be made using various methods. However, syringe-syringe or sonication methods are not recommended. These methods yield emulsions that are not stable enough to induce arthritis effectively. In addition, sonication cleaves collagen into fragments, which will be denatured at body temperature.

An electric homogenizer is highly recommended for preparing an emulsion:

1) Use a homogenizer (Figure 1) with a small blade (diameter of 5 mm or less) to emulsify the IFA (Catalog # 7002) with the collagen solution (Figure 2a). If the blade cannot reach the bottom of the mixing syringe, use a 5 ml or 10 ml syringe cut halfway from the plunger opening (Figure 2b). Seal the tip of the syringe with a 3-way stopcock. Next, clamp the syringe to a ring stand and place it in an ice water bath (Figure 3) to keep the emulsion cool during mixing, as heat will denature the collagen and fail to induce arthritis.

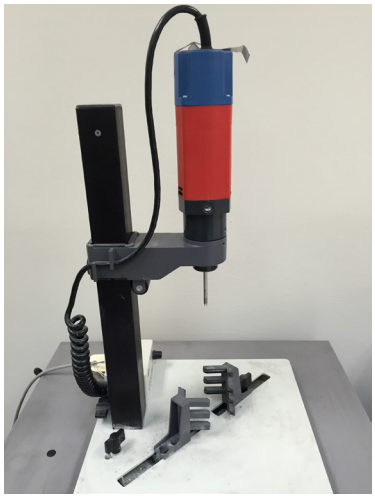

Figure 1 - Homogenizer (Virtis)

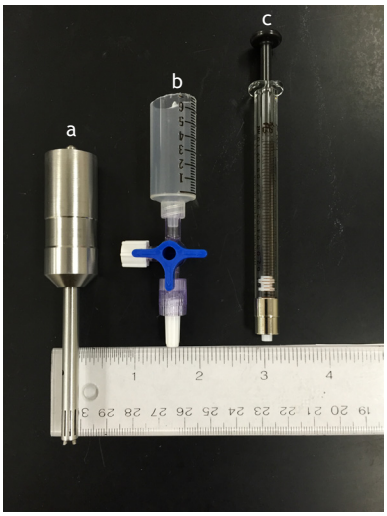

Figure 2 - Homogenizing blade - 0.5 cm diameter (a), Cut syringe - 10 ml with a 3-way stopcock (b), Hamilton glass syringe - 1 ml (c).

2607 151st Place NE
Redmond, WA 98052, USA
Phone: (425) 702-6365 or (888) 246-6373
Fax: (425) 882-3094
www.chondrex.com
support@chondrex.com

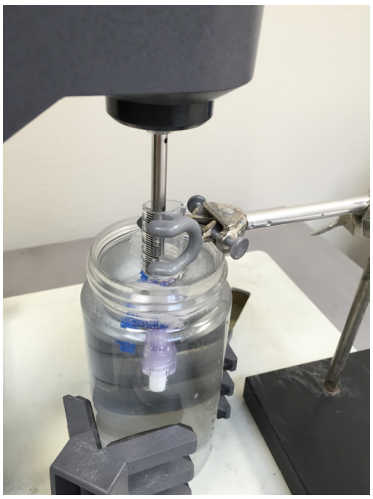

Figure 3 - A 10 ml syringe, which has been cut from the plunger end, clamped to a stand, and placed in an ice water bath.

Note: Seal the needle end with a 3-way stopcock.

2) Add one volume (maximum = 2.5 ml) of IFA to the syringe sealed with a 3-way stopcock. Then slowly add an equal volume of collagen solution (2 mg/ml in 0.05M acetic acid) drop-wise while mixing at low speed (1000-3000 rpm).

Note: To ensure a high quality emulsion, the maximum emulsion volume should be 5 ml. If more is needed, make several batches.

3) Continue mixing the emulsion at maximum speed (approximately 10,000 - 30,000 rpm) for 2 minutes. Cool down the emulsion by keeping the syringe in the ice water for 5 minutes. Repeat mixing and cooling 2-3 times. For larger volumes (2-5 ml), we suggest moving the blade throughout the emulsion while mixing for better uniformity.

4) Test the stability of the emulsion by adding one drop of emulsion into a beaker of water. If the emulsion is stable, the drop will remain as a solid clump which does not dissipate.

Note: If the emulsion dissipates onto the water surface then the emulsion is not stable. Add a few drops of adjuvant, mix again, and retest.

5) Transfer the emulsion to a 1 ml Hamilton glass syringe (Figure 2c). Injecting an accurate volume of emulsion is difficult with a plastic syringe.

Note 1: Remove air bubbles from the emulsion by forcefully swinging your arm towards the floor, with the Hamilton syringe in hand (plunger side down).

Note 2: Chondrex recommends injecting the collagen emulsion within an hour of preparation. Keep the emulsion cool at 4°C until use.

B. Injection Site

Place a 25 or 27 gauge x 5/8" needle on the Hamilton syringe. Before each injection, wipe the needle to prevent leakage of the emulsion. Insert the needle bevel side up and parallel to the tail at 2 cm from the base of the tail until the needle tip reaches 0.5 cm from the base. The entire needle should be subcutaneous. Inject 0.2 ml (200 μg collagen/rat) of the emulsion subcutaneously at the base of the tail (Figure 4). For a booster injection, insert the needle at 3 cm from the base of the tail until the tip reaches 1.5 cm from the base. The booster injection should be administered at a different location from the initial injection.

Note: Chondrex does not recommend subcutaneous injections in the back nor intraperitoneal (IP) injections, as emulsions cause severe inflammatory reactions in the peritoneal and thoracic cavities.

Figure 4 - Subcutaneous immunization of emulsion.

2607 151st Place NE
Redmond, WA 98052, USA

Phone: (425) 702-6365 or (888) 246-6373
Fax: (425) 882-3094

www.chondrex.com
support@chondrex.com

C. Immunization Schedule

There are several ways to induce arthritis with high incidence and high severity depending on the rat strain and the experimental purpose.

a) **Induction of arthritis by a single immunization without booster injection**:

Using the protocols for the preparation of emulsion (collagen-IFA) and injection site above, arthritis will develop 2-3 weeks after immunization depending on the strain. The incidence of arthritis should be 80-100% in high responder strains. The severity of arthritis should reach a score of 10-12 (maximum score 16) in highly susceptible strains such as BB rats.

b) **Induction of arthritis with a booster injection**:

To ensure a high incidence and severity of arthritis, a booster injection can be given on day 7 after the initial immunization. Prepare the collagen-IFA emulsion as described above and administer 0.1 ml of the emulsion subcutaneously in the tail. A rapid onset and severe arthritis are expected.

c) **Efficient induction of arthritis using synthetic muramyl dipeptide as an adjuvant**:

Since Complete Freund's adjuvant containing *M. tuberculosis* cannot be used to immunize rats with type II collagen, N-acetylmuramyl-L-alanyl-D-isoglutamine hydrate (MDP) can be used as an alternative adjuvant. Dissolve MDP at 4-8 mg/ml in distilled water. The collagen concentration should be 4 mg/ml in 0.05M acetic acid. Mix an equal volume of MDP and collagen solution before use. Make an emulsion by mixing the MDP-collagen solution with an equal volume of IFA as described above. Inject 0.2 ml of the emulsion subcutaneously in the tail. Arthritis incidence, severity, and consistency (from experiment to experiment) using this method may be greatly increased compared to the original protocol of using IFA alone (10).

Note: MDP alone induces arthritis at higher doses (~0.8 mg per rat). Thus, it is recommended to optimize the dose in individual strains of rats before using this protocol.

D. Onset of Arthritis

Clinically apparent arthritis with swollen joints appears 2-3 weeks after the first immunization. The onset of arthritis in BB rats tends to be earlier than other strains of rats, around 12-14 days. If immunization is effective, 100% of rats will develop arthritis in around 3 weeks.

E. Evaluating Arthritis

a) **Scoring:**

Disease can be assessed by a qualitative clinical score or by determining paw thickness using a thickness gauge, such as a Mitutoyo loop handle dial thickness gauge with a round disc. These methods are applicable for all arthritis models including classic CIA, adjuvant-induced arthritis, cotton-induced arthritis (15), and other inflammatory models. Chondrex provides a scoring system (Table 2) .

Table 2 - Qualitative scoring system used to assess severity of paw inflammation.

Score	Condition
0	Normal
1	Mild, but definite redness and swelling of the ankle or wrist, or apparent redness and swelling limited to individual digits, regardless of the number of affected digits
2	Moderate redness and swelling of ankle or wrist
3	Severe redness and swelling of the entire paw including digits
4	Maximally inflamed limb with involvement of multiple joints

b) **Paw volume assessment:**

At chosen time-points, the hind paw volume can be measured. Place a beaker containing a 10% soap solution of a known density on a top-loading balance and tare the balance to zero. Light anesthesia may facilitate this process. Immerse the limb in the solution to the level of the anatomical hair line. After retraction of the paw, the container is weighed, subtracted from the start weight and corrected for fluid density (17). Repeat the measurement four times.

2607 151st Place NE
Redmond, WA 98052, USA
Phone: (425) 702-6365 or (888) 246-6373
Fax: (425) 882-3094
www.chondrex.com
support@chondrex.com

c) **Serum analysis:**

High IgG autoantibody levels to type II collagen are important for inducing arthritis (8,12). More specifically, high levels of anti-type II collagen IgG2a and/or IgG2b subtype antibbodies are required for activation of complement, an essential step for inducing arthritis. Chondrex provides rat Anti-Collagen IgG and IgG subtype ELISA kits to analyze the antibody levels. Visit Chondrex.com for more information.

References

1. DE Trentham, AS Townes, AH Kang. Autoimmunity to type II collagen: an experimental model of arthritis. J Exp Med 146:857-68, 1977.

2. JS Courtenay, MJ Dallman, AD Dayan, A Martin, B Mosedale. Immunization against heterologous type II collagen induces arthritis in mice. Nature 283:666-668, 1980.

3. ES Cathcart, KC Hayes, WA Gonnerman, AA Lazzari, C Franzblau. Experimental arthritis in a nonhuman primate. I. Induction by bovine type II collagen. Lab Invest 54:26-31, 1986.

4. PH Wooley, et al. Type II collagen-induced arthritis in mice. IV. Variations in immunogenetic regulation provide evidence for multiple arthritogenic epitopes on the collagen molecule. J Immunol 135:2443-51, 1985.

5. MM Griffiths. Immunogenetics of collagen induced arthritis in rats. Intern Rev Immunology 4:1-15, 1988.

6. MM Griffiths, MA Cremer, DS Happer, S MaCall, GW Cannon. Immunogenetics of collagen induced arthritis in rats. Both MHC and non-MHC gene products determine the epitope specificity of immune response to bovine and chick type II collagens. J Immunol 149:309-316, 1992.

7. O Kohashi, J Kuwata, K Umehara, F Uemura, T Takahashi, A Ozawa. Susceptibility to adjuvant-induced arthritis among germ free, specific-pathogen free, and conventional rats. Microbiol Immunol 29:487-497, 1985.

8. PH Wooley. Collagen-induced arthritis in the mouse. Methods Enzymol 162:361-73, 1988.

9. WC Watson. Human HLA-DRb gene hypervariable region homology in the biobreeding BB rats: Selection of the diabetic-resistant subline as a rheumatoid arthritis research tool to characterize the immunopathologic response to human type II collagen. J Exp Med 155:1331-1339, 1990.

10. T Koga, S Sakamoto, K Onoue, S Kotani, A Sumiyoshi. Efficient induction of collagen arthritis by the use of a synthetic muramyl dipeptides. Arthritis Rheum 23:993-997,1980.

11. S Kleinau, H Erlandsson, L. Klareskog. Percutaneous exposure of adjuvant oil causes arthritis in DA rats. Clin Exp Immunol 96:281-284, 1994.

12. Y Shimozuru, et al. Collagen-induced arthritis in nonhuman primates: Multiple epitopes of type II collagen molecule can induce autoimmune mediated arthritis in outbred cynomolgus monkeys. Arthritis Rheum 41:507-514, 1998.

13. K Terato, et al. Collagen-induced arthritis in mice. Localization of an arthritogenic determinant to a fragment of the type II collagen molecule. J Exp Med 162:637-646, 1985.

14. E Michaelsson, et al. T cell recognition of carbohydrates on type II collagen. J Exp Med 180: 745-749, 1994.

15. CH Winter, EA Risley, GW Nuss. Carrageenan induced edema in hindpaw of the rat as an assay for antiinflammatory drugs. Proc Soc Exp Bio Med 111:544-547, 1962.

16. Breban MA1, Moreau MC, Fournier C, Ducluzeau R, Kahn MF. Influence of the bacterial flora on collagen-induced arthritis in susceptible and resistant strains of rats. Clin Exp Rheumatol. Jan-Feb;11(1):61-4, 1993.

17. Nielsen RH1, Christiansen C, Stolina M, Karsdal MA. Oestrogen exhibits type II collagen protective effects and attenuates collagen-induced arthritis in rats. Clin Exp Immunol. 2008 Apr;152(1):21-7, 2008.

2607 151st Place NE
Redmond, WA 98052, USA

Phone: (425) 702-6365 or (888) 246-6373
Fax: (425) 882-3094

www.chondrex.com
support@chondrex.com