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

Collagen-induced arthritis (CIA) in mice (1-3) shares immunological and pathological features with human rheumatoid arthritis (RA), and is the ideal model to study the pathogenesis of RA and to test therapeutics. Although the model is highly reproducible, certain considerations must be taken into account to successfully induce arthritis with sufficient incidence and severity. Therefore, a pilot study is recommended for first time users of this animal model.

1. Animal Vendors

From vendor to vendor and even within the same strain, the genetic background and bacteria flora will vary among mice. These differences affect how the mice will respond to various reagents, thus impacting the results of your experiments (39). Chondrex, Inc. recommends testing animals from different vendors using a defined protocol before proceeding with a full-scale experiment.

2. Housing Condition & Diet

Chondrex, Inc. recommends housing animals in Specific Pathogen Free (SPF) conditions rather than conventional conditions to avoid variability within experiments caused by bacterial and viral infections. For example, mice infected with mouse hepatitis virus (MHV) will not develop CIA (unpublished observation).

The incidence and severity of arthritis varies in mice fed with different commercially available rodent chows. The highest disease incidence has been observed in mice fed a high fat diet designed for breeders (Purina Mouse Chow 5015) (4).

3. Mouse Age & Strains

Mice should be at least 7-8 weeks old with a mature immune system. Aged mice may exhibit poor incidence and severity.

Susceptibility to CIA is linked to MHC-class II molecules which respond to individual species of type II collagen used for immunization (5). DBA/1 (H-2^q) and B10.RIII (H-2^r) mice are highly susceptible to CIA. DBA/1 mice respond to chick, bovine, and porcine type II collagen. B10.RIII mice respond to bovine and porcine type II collagen, but respond poorly to chick type II collagen.

DBA/1 (H-2^q) and B10.RIII (H-2^r) mice respond poorly to mouse type II collagen. Even after extensive immunization with mouse type II collagen, CIA incidence is still very low (approximately 10%) (6).

On the other hand, some CIA resistant mouse strains are capable of producing arthritogenic antibodies, suggesting that CIA is not only restricted by MHC types. For example, INF-γ or IL-10 knockout CIA resistant C57BL/6, 129/Sv (H-2^b), and Balb/c (H-2^d) mice can produce arthritogenic autoantibodies and develop arthritis. This indicates that susceptibility to arthritis is also highly regulated by cytokines (7).

A list of mouse strains commonly used for CIA and Collagen Antibody-Induced Arthritis (CAIA) are in Table 1.

Table 1 - Mouse strains commonly used for CIA and CAIA

Mouse Strain	H-2 Type	CIA Susceptibility	Reference	CAIA Susceptibility	Ref	Note
DBA/1	q	High	2, 4, 5	High	13, 20	INFγ high
B10.Q	q	High	5	(High)		
B10.G	q	High	5	(High)		
NFR/N	q	High	37	(High)		
SWR	q	Resistant	12	Resistant		C5 deficient
B10.RIII	r	High	5	High	13	Low response: chick and human type II
B10	b	Low	9	(High)		* Need alternative immunization
C57BL/6	b	Low	9	Moderate - High	8, 17, 29	LPS low responder – * Need alternative immunization
C57BL/6 beige	b	Resistant	19	Resistant		PMN mutation
C57BL/6 x 129/Sv	b	Low	9	Moderate - High	29, 30	* Need alternative immunization
129/Sv	b	Resistant	9	High	26	
B10.D2/nSn	d	Resistant	19	High	19	
B10.D2/oSn	d	Resistant	19	Resistant	19	C5 deficient
Balb/c	d	Resistant		High	13	
Balb/c nu/nu	d	Resistant		Resistant	27	B & T cell deficient
C3H/He	k	Low	38	(Low)		
B10.S	s	Resistant	5	?		
SJL/1	s	Moderate	2	(High)		
C.B-17 scid/scid		Resistant		High	17	B & T cell deficient

Parenthesis - assumed, but not tested

* Develops arthritis by alternative immunization with CFA containing high concentrations of *M. Tuberculosis*

4. Adjuvant

Complete Freund's Adjuvant (CFA), consisting of high quality *M. tuberculosis*, is essential to induce severe arthritis in mice because it induces a strong immune response. Unlike rats, mice will not develop arthritis by immunizing with type II collagen emulsified with Incomplete Freund's Adjuvant (IFA).

A strong antibody response as well as the correct antibody subtype is critical for inducing arthritis in mice. Antibody production depends on the concentration of *M. tuberculosis* in CFA, and sufficient anti-collagen IgG2a and IgG2b subtype levels are necessary to activate complement, an essential step for inducing arthritis (8). In fact, Campbell, *et al.* reported that CFA containing 5 mg/ml of *M. tuberculosis* successfully induced arthritis with high incidence (50-70%) in CIA resistant mouse strains, such as C57BL/6, B10, and 129/Sv mice (H-2^b) (9).

However, because high concentrations of *M. tuberculosis* induce severe inflammation, contact your institution's animal committee for choosing the appropriate CFA. The following is a list of adjuvants provided by Chondrex, Inc.

Catalog #	Description
7002	Incomplete Freund's Adjuvant, 5 ml
7008	Complete Freund's Adjuvant, 5 ml x 1 mg/ml
7009	Complete Freund's Adjuvant, 5 ml x 2 mg/ml
7015	Complete Freund's Adjuvant, 5 ml x 3 mg/ml
7001	Complete Freund's Adjuvant, 5 ml x 4 mg/ml
7023	Complete Freund's Adjuvant, 5 ml x 5 mg/ml

5. Collagen

Highly purified native type II collagen must be used. Deglycosylation and denaturation of collagen will affect arthritogenicity (10) and minor contaminants such as pepsin may yield false positive reactions in a T-cell stimulation assay (11).

Chondrex, Inc. offers immunization grade type II collagen for the CIA model depending on the mouse strain (please see table 1 for more information). For example, DBA/1 mice strongly respond to chick or bovine type II collagen, whereas C57BL/6 mice strongly respond to chick 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

Lyophilized collagen and collagen in acid solution are stable at -20°C in the dark. Collagen should be dissolved at 2 mg/ml in 0.05M acetic acid and gently stirred overnight at 4°C. The collagen solutions can be kept at 4°C for one week, but should then be kept at -20°C thereafter.

Protocol to Induce Arthritis

A. Emulsion Preparation

The emulsion quality is critical for inducing arthritis with high incidence and severity. Emulsions can be made using various methods. However, syringe-syringe and sonication methods are not recommended because emulsions prepared by these methods are unstable and do not induce arthritis effectively. In addition, sonication cleaves collagen into fragments which reduces its arthritogenicity.

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 CFA (IFA for booster injection) 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 which will fail to induce arthritis.
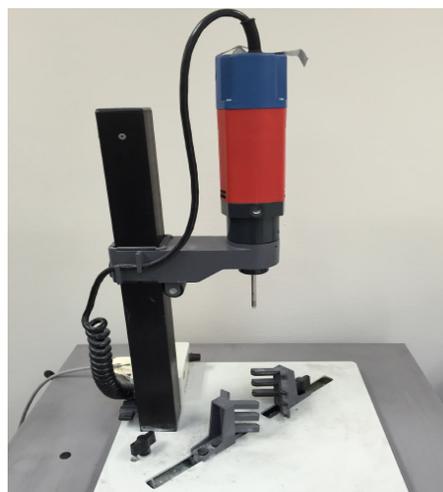

Figure 1 - Homogenizer (Virtis)

2607 151st Place NE
Redmond, WA 98052, USA

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

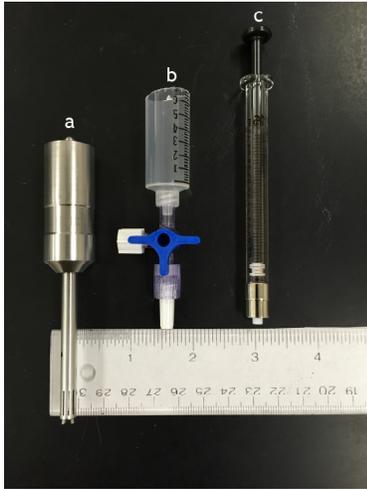www.chondrex.com
support@chondrex.com

Figure 2 - (a) Homogenizing blade - 5 mm diameter (b) Cut syringe - 10 ml with a 3-way stopcock (c) Hamilton glass syringe - 1 ml

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 syringe tip with a 3-way stopcock.

2)   Add one volume (maximum = 2.5 ml) of CFA (IFA for booster injection) into the syringe. 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 on 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: We recommend 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 0.1 ml (100 μg collagen/mouse) 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. Subcutaneous injections into the back are not recommended, as the incidence of arthritis is low.

Note: We do not recommend intraperitoneal (IP) injections, since both CFA and IFA cause severe inflammatory reactions in the peritoneal and thoracic cavities.

Figure 4 - Subcutaneous injection 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

Chondrex, Inc.

C. Immunization Schedule

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

a) **Induction of arthritis by a single immunization without booster injection in <u>high responder</u> strains**:

Inject the emulsion of collagen and CFA containing a final concentration of 2 mg/ml of *M. tuberculosis* in mice. Arthritis will develop 28-35 days after immunization in CIA high responder strains, such as DBA/1J (H-2^q) and B10.RIII (H-2^r) mice. The incidence of arthritis should be 90-100% at 42-56 days. The severity of arthritis should be high and reach a score of 10-12 (maximum score 16).

Note: Inflammation at the injection site is generally severe because of the high concentration of *M. tuberculosis*. Thus, some facilities may not accept this protocol. In this case, use one of the following protocols (b) or (d).

b) **Induction of arthritis with a booster injection in <u>high responder</u> strains**:

Inject the emulsion of collagen and CFA containing a final concentration of 0.5 mg/ml of *M. tuberculosis*. Administer a booster injection with an emulsion of collagen and IFA on day 21. The booster injection should be administered at a different location than the first injection site. Arthritis will develop 28-35 days after the first immunization. The incidence of arthritis is around 80-100% and severity of arthritis will reach scores of 8-12 (maximum score 16) at 42-56 days.

c) **Induction of arthritis by an alternative immunization protocol with high *M. tuberculosis*-content adjuvant in <u>low responder</u> strains**:

CIA can be induced in several CIA low responder mouse strains such as B10 (H-2^b), C57BL/6 (H-2^b), and C57BL/6x129/Sv (H-2^b). Inject the emulsion of collagen and CFA containing a final concentration of 2.5 mg/ml of *M. tuberculosis*. Administer a booster injection with an emulsion of collagen and CFA containing a final concentration fo 2.5 mg/ml of *M. tuberculosis* on day 21. Arthritis will develop in 28-35 days after the first immunization. The maximum incidence of arthritis in these mice is approximately 50-70% at 42-56 days (9).

Note: The inflammatory reaction at the injection site might be very severe, thus some animal committees may not accept this protocol. An alternative mouse arthritis model with reduced inflammation (at the injection site), as well as dramatically shorter experimental times is the collagen antibody-induced arthritis (CAIA) model. Chondrex, Inc.'s anti-type II collagen monoclonal antibody cocktail (Arthrogen-CIA®) and LPS

will induce arthritis in these CIA resistant mouse strains. Visit www.chondrex.com for more information.

d) **Synchronizing onset of arthritis by LPS injection**:

LPS has a synergistic effect in triggering arthritis with sub-arthritogenic levels of autoantibodies to type II collagen (13). Furthermore, severity and incidence in CIA can be increased by an administration of LPS (a B-cell mitogen) (14), *Mycoplasma arthritidis* (a T cell mitogen) (MAM)(15), and *Staphylococcal* enterotoxin B (SEB)(16). These bacterial toxins can be used not only to trigger and enhance arthritis, but also to synchronize the onset of arthritis.

For this protocol, inject the emulsion of collagen and CFA containing a final concentration of 0.5 mg/ml of *M. tuberculosis* according to protocol (b). Inject LPS (25-50 μg in saline) intraperitoneally on day 25-28 or 3-5 days before the desired onset of arthritis. Arthritis will develop within 24-48 hours in 90-100% of mice.

Note: Mice immunized with CFA develop severe immune-suppression for 2-4 weeks following the first immunization. Therefore, some mice will be highly susceptible to LPS injection (50 μg). As previously mentioned (see Animal Vendors), Chondrex, Inc. suggests testing animals from different vendors before proceeding with a full-scale experiment.

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

D. Evaluating Arthritis

a) Scoring:

Disease can be assessed by 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, CAIA, and other inflammatory models. Chondrex, Inc. provides a scoring system (Table 2) and supplemental flyer (visit www.chondrex.com).

Note: Mouse paw volume cannot be determined by a plethysmograph as used for rat paw volume measurement because the mouse paw is too small.

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) Serum Analysis:

High IgG autoantibody levels to type II collagen, as well as the antibody subtype are important for inducing arthritis (8,12). More specifically, since IgG2a and IgG2b activate complement, the antibody levels are an essential step for inducing arthritis. Chondrex, Inc. provides Anti-Collagen IgG and IgG subtype ELISA kits to analyze the antibody levels. Visit www.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. Collagen-induced arthritis in the mouse. Methods Enzymol 162:361-373, 1988.

5. 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 135:2443-2451, 1985.

6. R Holmdahl, L Jansson, E Larsson, K Rubin, L Klareskog. Homologous type II collagen induces chronic and progressive arthritis in mice. Arthritis Rheum 29:106, 1986.

7. RA Ortmann and EM Shevach. Susceptibility to collagen-induced arthritis: cytokine-mediated regulation. Clin Immunol 98:109-118, 2001.

8. WC Watson, AS Townes. Genetic susceptibility to murine collagen II autoimmune arthritis. Proposed relationship to the IgG2 autoantibody subclass response, complement C5, major histocompatability complex (MHC) and non-MHC loci. J Exp Med 162:1878-1891, 1985.

9. IK Campbell, JA Hamilton, IP Wicks. Collagen-induced arthritis in C57BL/6 (H-2b) mice: new insights into an important disease model of rheumatoid arthritis. Eur J Immunol 30:1568-1575, 2000.

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

11. M Anderson, R Holmdahl. Analysis of type II collagen reactive T cells in the mouse. I. Different regulation of autoreactive vs. non-autoreactive anti-type II collagen T cells in the DBA/1 mouse. Eur J Immunol 20:1061-1066, 1990.

12. R Reife, N Loutis, W Watson, K Hasty, J Stuart. SWR mice are resistant to collagen-induced arthritis but produce potentially arthritogenic antibodies. Arthritis Rheum 34:776-781, 1991.

13. K Terato, et al. Collagen-induced arthritis in mice: synergistic effect of E.coli lipopolysaccharide bypasses epitope specificity in the induction of arthritis with monoclonal antibodies to type II collagen. Autoimmunity 22:137-147, 1995.

14. S Yoshino, E Sasatomi, Y Mori. Oral administration of lipopolysaccharide exacerbates collagen-induced arthritis in mice. J Immunol 163:3417-3422, 2000.

15. BC Cole, MM. Griffiths. Triggering and exacerbation of autoimmune arthritis by the Mycoplasma Arthritidis superantigen MAM. Arthritis Rheum 36:994-1002, 1993.

16. Y Takaoka, H Nagai, M Tanahashi, K Kawada. Cyclosporin A and FK-506 inhibit development of superantigen-potentiated collagen-induced arthritis. Gen Pharmcol 30:777-782, 1998.

17. T Kagari, H Doi and T Shimozato. The importance of IL-1β and TNF-α, and the noninvolvement of IL-6, in the development of monoclonal antibody-induced arthritis. J Immunol 169:1459-1466, 2002.

18. JM Stuart, MA Cremer, AS Townes, AH Kang. Type II collagen-induced arthritis in rats: passive transfer with serum and evidence that IgG anticollagen antibodies can cause arthritis. J Exp Med 155:1- 16, 1982.

19. WC Watson, PS Brown, JA Pitcock, AS Townes. Passive transfer studies with type II collagen antibody in B10.D2/ old and new line and C57BL/6 normal and beige (Chediak-Higashi) strains: evidence of important roles for C5 and multiple inflammatory cell types in the development of erosive arthritis. Arthritis Rheum 30:460-5, 1987.

20. K Terato, et al. Induction of arthritis with monoclonal antibodies to collagen. J Immunol 148:2103-2108, 1992.

21. 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.

22. LK Myers, H Miyahara, K Terato, JM Seyer, AH Kang. Collagen-induced arthritis in B10.RIII mice (H-2r): identification of an arthritogenic T cell determinant. Immunol 84:509-513, 1995.

23. K Terato, et al. Induction of chronic autoimmune arthritis in DBA/1 mice by oral administration of type II collagen and Escherichia Coli Lipopolysaccharide. British J Rheum 35:828-838,1996.

24. P Wallace, et al. Regulation of inflammatory responses by Oncostatin M. J Immunol 162:5547- 5555, 1999.

25. A de Fougerolles, et al. Regulation of inflammation by collagen-binding integrins α1β1 and α2β1 in models of hypersensitivity and arthritis. J Clin Invest. 105:721-729, 2000.

26. FS Larox, et al. A novel model of polyarthritis induced in mice using monoclonal antibodies to type II collagen. Characterization and effects of chemically modified tetracycline. Arthritis Rheum 42:s121 (supplement).

27. Y Tsuchiya, M Maeda, K Hanada, et al. Mouse arthritis model induced by anti-type II collagen monoclonal antibody cocktail: Difference in distribution of diseased joints by strain and immunodeficient status. Proc Japanese Society Animal Models for Human Diseases.19:14-22, 2003.

28. K Takagishi, et al. Serum transfer of collagen arthritis in congenitally athymic nude rats. J Immunol 134:3864-3867, 1985.

29. H Kato, et al. Effect of NOS2 gene deficiency on the development of autoantibody mediated arthritis and subsequent articular cartilage degeneration. J Rheumatology 30:247-255, 2003.

30. K Yumoto, et al. Osteopontin deficiency protects joints against destruction in anti-type II collagen antibody-induced arthritis in mice. PNAS 99: 4556- 45561, 2002.

31. LK Myers, et al. The genetic ablation of cyclooxigenase 2 prevents the development of autoimmune arthritis. Arthritis Rheum 43:2687- 2693, 2000.

32. T Itoh, et al. The role of matrix metalloproteinase-2 and metalloproteinase-9 in antibody-induced arthritis. J Immunol 169:2643-2647, 2002.

33. JM Labasi, et al. Absence of the P2X7 receptor alters leukocyte function and attenuates an inflammatory response. J Immunol 168:6436-6445, 2002.

34. Z Han, et al. Joint damage and inflammation in c-Jun N-terminal kinase 2 knockout mice with passive murine collagen-induced arthritis. Arthritis Rheum 818-823, 2002.

35. JM McCoy, et al. The role of prostaglandin E2 receptor in the pathogenesis of rheumatoid arthritis. J Clin Invest 110:651-658, 2002.

36. RC Newton, et al. Biology of TACE inhibitor. Ann Rheum Dis 60:iii25-iii32, 2001.

37. R Holmdahl, L Jannson, M Anderson, et al. Immunogenetics of type II collagen autoimmunity and susceptibility to collagen arthritis. Immunology 65:305-10, 1988.

38. S Thornton, G Boivin, KN Kim, et al. Heterogeneous effect of IL-2 on collagen-induced arthritis. J Immunol 165:1557-1563, 2000.

39. Ivanov II. et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 139 (3): 485-498, 2009.

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