Collagen Antibody Induced Arthritis (CAIA) in Mice

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COLLAGEN ANTIBODY INDUCED ARTHRITIS (CAIA) MODEL

Collagen-induced arthritis (CIA) has been extensively used as a mouse model for rheumatoid arthritis (RA). However, the CIA model requires at least 6-8 weeks to complete a typical study (1-12). To reduce this timeline, Chondrex, Inc. has developed a rapid arthritis model using monoclonal antibodies (mAbs) against type II collagen. By injecting the mAbs into mice, arthritis can be induced within a few days. Additional advantages of this CAIA model include: 1) inducing arthritis in various mouse strains, not just CIA susceptible mice, 2) quick screening and evaluation of anti-inflammatory compounds, 3) studying the roles of individual genes and their products in arthritis development using gene knockout and transgenic mice, and 4) studying various relevant inflammatory mediators and factors such as bacterial and viral toxins which may be involved in human RA as described (13-17).

CIA is mediated by autoantibodies to type II collagen and complement, thus arthritis can be induced by administering polyclonal antibodies (18-19) or a specific combination of monoclonal antibodies to type II collagen (mAb cocktail) (20-21). These autoreactive antibodies recognize specific antigenic determinants (arthritogenic epitopes) located in the cyanogen bromide-cleaved fragment 11 (CB11) or CB8 fragment of mouse type II collagen, depending on the MHC type (22-23).

Chondrex, Inc.'s mAb cocktail to induce mouse arthritis is a mixture of five unique mAbs: A2-10 (IgG2a), F10-21 (IgG2a), D8-6 (IgG2a), D1-2G (IgG2b), and D2-112 (IgG2b). Two mAbs (F10-21 and D8-6) recognize individual epitopes clustered within the 83 amino acid peptide fragment of LysC-2 (291-374) which is prepared by Endoproteinase LysC digestion, and three mAbs (A2-10, D1-2G, and D2-112) recognize the 167 amino acid peptide fragment of LysC-1 (124-290) of the CB11 fragment (124-402) of mouse type II collagen (13). These epitopes are highly conserved amino acid sequences in many different species including chicken, mouse, rat, bovine, porcine, monkey, and human (20-21).

Severe and consistent arthritis can be induced in mice by combining a sub-arthritogenic dose of the mAb cocktail and Lipopolysaccharide (LPS) from *Escherichia coli (E. coli)*: O111:B4(13). This model was developed based on the hypothesis that bacterial toxin(s), such as LPS, absorbed through the gastrointestinal tract play a synergistic and pathological role with sub-arthritogenic levels of autoantibodies to type II collagen, triggering arthritis (24). The advantages of this model over the classical CIA model are multifold. First, synchronized arthritis is induced within a few days with nearly 100% incidence. Second, a variety of mouse strains such as CIA-resistant, T-cell deficient, knockout, and transgenic mice can be used (see Table 1).

A typical time course study of the CIA and CAIA models are shown in Figure 1. The CAIA model can reduce the timeline of experiments down to a tenth of that of the CIA model.

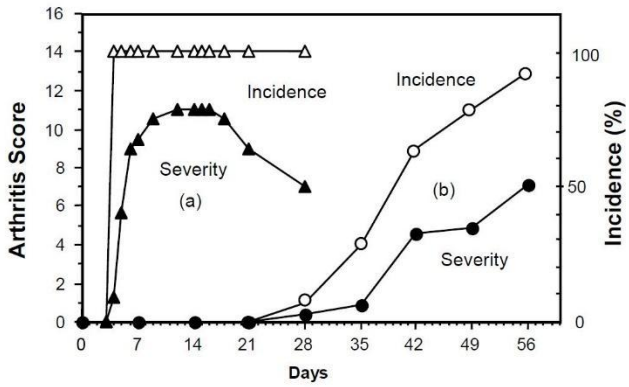

Figure 1 - CAIA vs. CIA: (a) Triangles: Arthritis developed in 100% of mice within 24-48 hours after LPS injection and reached a maximum score within 5-7 days in the CAIA model. (b) Circles: in the CIA model, it took 4 weeks for the onset of arthritis even in CIA highly susceptible strains such as DBA/1J and B10.RIII mice.

Solid markers = Arthritis score (severity of arthritis)

Blank markers = Incidence of arthritis (%).

Animal Care and Diet

It is imperative to use 7-8-week-old mice for the CAIA model. Older mice may demonstrate lower incidence and disease severity. Specific pathogen free (SPF) housing conditions are recommended over conventional housing conditions, as bacterial contamination may reduce the immune response, resulting in attenuated arthritis. Intestinal bacteria flora varies depending on animal vendors and can affect the host immune system's susceptibility to antigens such as LPS. Some mice are highly susceptible to LPS and killed by LPS injections. Chondrex, Inc. recommends testing mice for LPS susceptibility prior to running large studies. Diet considerations for mice are not necessary, however, Chondrex, Inc. recommends a high fat diet (Purina Mouse Chow 5015) for mouse inflammation studies (4). Please contact customer support at support@chondrex.com for guidance regarding animals and housing conditions.

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16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

Mouse Strains

Administering the anti-type II collagen mAb cocktail bypasses the host's need to generate autoantibodies to type II collagen. Thus, arthritis can be induced in mouse strains which lack CIA-susceptible MHC haplotypes (i.e. H-2q and H-2r). All mouse strains with normal inflammatory responses including complement activation should be susceptible to CAIA. Table 1 lists the mouse strains that have been tested to date.

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

(Parentheses-assumed, but not yet tested)

*Develops arthritis by alternative immunization with Complete Freund's Adjuvant containing 5 mg/ml *M. tuberculosis*

The following gene knockout mice have been used to study the role of genes in the CAIA model (29-39).

1) NOS2 knockout mice (30)
2) Osteopontin knockout mice (31)
3) COX-1 and COX-2 knockout mice (32)
4) MMP-2 (gelatinase A) and MMP-3 (gelatinase B) knockout mice (33)
5) $P2X_7$ receptor knockout mice (34)
6) c-Jun N-Terminal Kinase knockout mice (35)
7) Prostaglandin E2 receptor knockout mice (36)
8) CD69 null mice (37)

DBA/1 (H-2q) and B10.RIII (H-2r) mice are susceptible to both CIA and CAIA (13).

Balb/c (H-2d) mice, which are resistant to CIA, are highly susceptible to CAIA and are the most commonly used strain (13, 25-27), being readily available.

C.B-17 scid/scid mice are T-cell deficient mice which can be used for CAIA as T-cells are not required for antigen recognition for antibody production in arthritis development (17). These mice may even develop more severe arthritis than T-cell normal strains (unpublished data), as T-cells may play roles in down-regulating inflammation during healing as well as in up-regulating inflammatory reactions.

SWR (H-2q) mice have a CIA-susceptible haplotype but are resistant to CIA and CAIA due to a C5 deficiency. Other C5 deficient mouse strains, B10.D2/oSn and NOD/LtSz scid/scid, are also apparently resistant to CAIA (12,19,27).

Nude mice are resistant to CAIA (28) for unknown reasons despite nude rats being highly susceptible to antibody-induced arthritis (29). Nude mice may lack select pro-inflammatory cytokine expression.

C57BL/6 mice are most commonly used as parent mice for gene knockout and transgenic mice. Although wild type C57BL/6 mice are apparently low responders to LPS, these mice also develop severe arthritis in CAIA with LPS. However, a higher dose of the mAb cocktail is required such as 5 mg/mouse instead of 1.5 mg/mouse (21, 30). C57BL/6-backcrossed to 129/Sv mice are commonly used for creating gene knockout mice. Some of these backcrossed mice respond to LPS, thus severe arthritis can be induced with an ordinary 1.5 mg/mouse dose (30-31).

PROTOCOL TO INDUCE CAIA

Chondrex, Inc. recommends administering the mAb cocktail intravenously (i.e. tail vein). Intraperitoneal (IP) injection can also be used in CAIA susceptible strains; however, the severity of arthritis tends to be lower and the period of active inflammatory arthritis shorter. Individual investigators must determine the best protocol for their own experimental purposes.

Bacterial toxins such as LPS (B-cell mitogen), Staphylococcal enterotoxin B (T-cell mitogen), and *Mycoplasma arthritidis* mitogen (T-cell mitogen) act synergistically with antibodies to type II collagen in arthritis development. Thus, the mAb dose required for inducing arthritis can be reduced in the presence of these toxins. For example, injecting 1.5 mg/mouse of the mAb cocktail on day 0 followed by an LPS injection (25-50 μg) on day 3 can induce arthritis in nearly 100% of CAIA susceptible mice, such as Balb/c, DBA/1, B10.RIII, and C.B-17 scid/scid mice. Inject 5 mg/mouse for CAIA susceptible C57BL/6 mice. Arthritis progresses rapidly, and acute inflammation peaks on day 7-10 and persists for 2 weeks.

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Arthritis can be further exacerbated by an additional LPS injection (25-50 µg) on day 10 or 14. The resulting joint destruction is persistent, leading to ankylosis even though active inflammation declines after 3 weeks. Table 3 references compounds which have successfully blocked inflammation in the CAIA model.

Table 2. Arthrogen-CIA Arthritogenic Monoclonal Antibody 5-Clone Cocktail Kit

Catalog Number	Quantity
53100	mAb Cocktail: 10 mg/ml x 10 ml LPS: 0.5 mg/ml x 7.5 ml
53040	mAb Cocktail: 10 mg/ml x 4 ml LPS: 0.5 mg/ml x 3.5 ml
53010	mAb Cocktail: 10 mg/ml x 1 ml LPS: 0.5 mg/ml x 1.5 ml

The optimal dosages for mAb cocktails and LPS depend on several factors, including the mouse strain, animal vendor, and housing environment.

1) High doses of LPS, even as low as 50 µg per mouse, can be lethal depending on the health of the animals. Chondrex, Inc. recommends performing a preliminary pilot study using a small number of mice to test LPS dosages of 10, 25, and 50 µg without any antibody administration.

2) Susceptibility to CAIA also varies significantly. A pilot study is recommended to identify the ideal dose to ensure the desired disease severity before proceeding with a large-scale study.

Table 3 - Compounds that Have Successfully Blocked Inflammation in the mAb-Induced Arthritis Model.

Compound	Class	Reference
Oncostatin M	Cytokine	25
Anti-Integrins α1β1, 2β1	Adhesion Molecule	26
Chemically Modified Tetracycline (CollaGenex)	Matrix Inhibitor Metalloproteinase	27
Anti-CD44 (IM7 Clone)	Adhesion Molecule	Unpublished Data
Dexamethasone (0.15 –0.5 mg/kg)	Glucocorticoid	Unpublished Data
Anti-IL-1β Antibody	Cytokine	17
Anti-TNF-α Antibody	Cytokine	17
Anti-MIP-1α Antibody	Cytokine	17
TACE Inhibitor	TNF Converting Enzyme Inhibitor	37
Methotrexate (0.25 mg/kg, single IM injection) - Effective in p53 knockout mice - Not effective in Balb/c mice	Anti-proliferation Communication	Unpublished Data

EVALUATING ARTHRITIS

Disease can be assessed by qualitative clinical score or by determining paw thickness using a Mitutoyo loop handle dial thickness gauge with a round disc. Unlike rat paw volume, mouse paw volume cannot be determined with a plethysmograph because the mouse paw is too small. Chondrex, Inc. provides a scoring system (Table 2) and a supplemental flyer (please visit www.chondrex.com for more information).

Table 4 - Qualitative Scoring System 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 involving multiple joints

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16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com