

MD Biosciences

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FOR RESEARCH USE ONLY

Arthrogen-CIA[®] Collagen

Immunization Grade Type II Collagen and Adjuvant

Collagen-Induced Arthritis (CIA)

Autoimmune-mediated polyarthritis can be induced in certain strains of rodents and non-human primates by immunizing them with native type II collagen. This collagen-induced arthritis (CIA) model is a widely used and well characterized model for studying the pathogenesis of human rheumatoid arthritis and for testing and evaluating therapeutics.

Susceptibility to CIA is linked to MHC-class II molecules but also depends on the species of type II collagen used for immunization (see Table 1 below). Importantly, heterologous type II collagen is more immunogenic and arthritogenic than homologous type II collagen in most animals. However, a recent study shows that CIA can be induced in CIA resistant strains of mice such as C57BL/6 (H-2b) and Balb/c (H-2d) in which either INF γ or IL-10 signaling has been abolished by gene deletion. These observations strongly indicate that non-MHC genes play important regulatory roles in inflammatory arthritis. However, it is also important to notice that these CIA resistant wild type strains develop severe arthritis by antibody transfer: for example by a cocktail of monoclonal antibodies to type II collagen (see NOTE). Thus, it cannot be ruled out that antibodies produced in CIA-resistant strains differ in the epitope specificity and thus are less arthritogenic compared to antibodies produced in these CIA susceptible strains.

Table 1: Susceptibility to CIA in mice, rats, and monkeys depends on both MHC haplotypes and species of type II collagen used for immunization

| Species of Type II Collagen | Mouse | | Rat | | Primate Monkeys (outbred) |
|-----------------------------|--------------|-----------------|------------|--------------|---------------------------|
| | DBA/1 (H-2q) | B10.RIII (H-2r) | LOU (RT1u) | Lewis (RT1I) | |
| Chicken | +++ | - | +++ | ++ | +++ |
| Bovine | +++ | +++ | +++ | +++ | +++ |
| Porcine | +++ | +++ | +++ | +++ | nd |
| Human | ++ | - | + | + | ++ |

Five species (chicken, bovine, porcine, rat and human) of type II collagen used for the induction of arthritis in a variety of animals are provided. Arthrogen-CIA[®] Type II collagen is supplied lyophilized and in solution. In addition, an optimized adjuvant for the induction of CIA in mice is also available upon request.

NOTE: C57Bl/6 mice and C57Bl/6 back-crossed with other strains that are commonly used for transgene and gene knockout experiments are resistant to authentic CIA. However, these mice are generally susceptible to antibody-transfer arthritis, if their complement levels are normal. Thus, we recommend the use of Arthritogen-CIA[®] monoclonal antibody cocktail for inducing arthritis in these mice.

Protocol for the Induction of CIA in Mice

DBA/1 (H-2^d), B10.RIII (H-2^f) and their congenic mice are susceptible to CIA. Young (7 – 8 week old) and healthy mice raised under SPF conditions should be used. Old mice (more than 6 months old) also develop arthritis, however the onset and severity of arthritis generally varies in these mice compared to young ones. Both male and female mice can be used. The type of adjuvant and the stability of the emulsion are equally important in the induction of arthritis. Therefore, Arthrogen-CIA[®] adjuvant, an optimized adjuvant, is highly recommended.

1. Preparation of Emulsion

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1. Dissolve type II collagen at 2.0 mg/ml in 0.05M acetic acid overnight at 4°C (**collagen must be kept cold to prevent denaturation**) with constant, but gently stirring.

2. Emulsify the collagen in the adjuvant at a 1:1 ratio as follows: Add 1 volume of the Arthrogen-CIA® adjuvant in a 5 or 10 ml syringe with a T-connector. Using a homogenizer (such as a Virtis), begin to mix

the adjuvant at slow speed. Add an equal volume of collagen solution **dropwise**, to the mixing adjuvant. Continue to mix until a stiff emulsion results (approximately 2-3 minutes for smaller volumes or 3-5 minutes for larger volumes) at maximum speed).

NOTE: Do not attempt to make more than 6 ml of emulsion at one time. The syringe should be placed in a plastic beaker containing ice water to prevent heating and denaturation of the collagen during mixing. If a stable emulsion is not formed, add a few drops of adjuvant and mix again.

2. Immunization

Transfer the emulsion via the T-connector to a 1.0 ml Hamilton glass syringe outfitted with a 29 or 30 gauge needle or use a 1.0 ml tuberculin syringe. Inject each mouse approximately 1 – 2 cm from the base of the tail with 0.05 – 0.1 ml of the emulsion, subcutaneously (**this is critical**). A whitish bolus beneath the dermis should be visible.

NOTE: In general, a booster injection will be not required for the induction of arthritis. However, if it is necessary, the collagen should be emulsified in Incomplete Freund's Adjuvant (ICFA) as described above. The boost (0.05 – 0.10 ml of collagen emulsion) should be given at day 7 or 21 post-immunization.

3. Onset and Severity of Arthritis

High CIA-susceptible mice such as DBA/1 and B10.RIII generally develop arthritis 4 – 5 weeks after immunization with Arthrogen-CIA® type II collagen. Fully developed arthritis, including red and swollen paws, will be observed 3-5 days after the onset. Active, inflammatory arthritis lasts more than 3 – 4 weeks. Although inflammation will eventually

subside, joints will never heal and ankylosis will be permanent.

For more detailed information on the induction of arthritis.

Induction of CIA in Rats

Rats are generally susceptible to adjuvant arthritis. To avoid the development of **adjuvant arthritis**, ICFA must be used when preparing the type II collagen emulsion. Inject 0.1 ml of collagen emulsified with ICFA at the base of the tail. Onset of CIA in highly susceptible strains of rats such as BB rats is generally 10 – 14 days after a single immunization of type II collagen, and lasts more than 4 weeks.

Induction of CIA in Monkeys

Use Complete Freund's Adjuvant (heat-killed Mycobacteria Tuberculosis: 1mg/ml) to emulsify the collagen since monkeys are highly susceptible to M. Tuberculosis and develop severe inflammation at the site of injection as well as systemic myositis.

In general, 1 – 4 mg of type II collagen has been used for immunization, however, 0.25 mg of collagen can also induce arthritis. Inject the collagen emulsion into 10-20 sites in the back, intradermally. Onset of arthritis is generally 3-6 weeks after a single immunization of type II collagen, and lasts for 1 – 6 months depending on the individual animal.

Unfortunately, there is no definite method to predict which animals are susceptible or resistant to CIA. In general, the development of arthritis correlates with serum IgG antibody levels to autologous monkey type II collagen.