A Protocol for Successfully Inducing Collagen-Induced Arthritis (CIA) in Mice
胶原蛋白诱导小鼠关节炎实验方案

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（仅供科研使用，不用于临床诊断治疗）

BACKGROUND
背景知识 0

Collagen-induced arthritis (CIA) in mice shares immunological and pathological features with human rheumatoid arthritis (RA). The CIA model is ideal to study the pathogenesis of RA and to test therapeutics (1-3). 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.

胶原蛋白诱发(CIA) 的小鼠关节炎与人类类风湿关节炎具有共同的免疫学和病理学特征。因此CIA模型被广泛应用于研究致病机理和治疗方案的研究 （1-3）。尽管该模型具有高度可重复性，但要成功诱导高发病率和严重程度的关节炎，一些影响造模的因素需要考虑。以下是成功诱发小鼠关节炎的一些重要因素。因此，建议首次造模者需要考虑这些因素，确定可行性方案。

A. Animal Vendors
动物来源

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 experimental results (4). Chondrex, Inc. recommends testing animals from different vendors using a defined protocol before proceeding with a full-scale experiment.

即使同样品系的小鼠来自不同的供应商也可能会造成实验结果的差异，Chondrex公司建议开展实验前先测试不同供应商提供的小鼠。

B. Housing Condition & Diet
饲养条件和饮食

Chondrex, Inc. 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 For example, mice infected with mouse hepatitis virus (MHV) will not develop CIA (unpublished observation).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) (5).

为避免细菌和病毒感染在实验中引起的差异，Chondrex公司建议在无特定病原体 (SPF级) 条件下饲养动。 一般来说，对于肠道菌群，不管是否致病都显著影响宿主对抗原的免疫应答。 例如，对于感染肝炎病毒（MHV）的小鼠，胶原蛋白不能诱发关节炎。饮食也会影响关节炎的发病率和严重程度， CIA 在饲喂不同饲料的小鼠中差异很大。饲喂高脂肪的食物更容易引起高发病率关节炎（5）。

C. Mouse Age & Strains
小鼠年龄和品系

Mice should be at least 7-8 weeks old with a mature immune system. Aged mice may exhibit poor incidence and severity. Chondrex, Inc recommends consistently using the same age for better reproducibility in repeat studies. Susceptibility to CIA is linked to MHC-class II molecules which respond to individual species of type II collagen used for immunization (6). 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%) (7).

建议使用具有成熟免疫系统的7-8周龄的小鼠。老龄小鼠可能对CIA的易感性较差。Chondrex公司建议在重复实验中使用相同周龄的小鼠。小鼠对CIA的易感性与MHC-II 类分子相关（6）， 同时也取决于接种二型胶原蛋白的小鼠品系。DBA/1(H-2^q) 和 B10.RIII(H-2^r) 品系的小鼠被广泛应用，因为这两个品系对CIA高度易感。DBA/1(H-2^q)小鼠，对鸡，牛和猪二型胶原有免疫应答。B10.RIII小鼠对牛和猪二型胶原有免疫应答，但对鸡而型胶原的免疫应答较弱。两种品系小鼠对小鼠的二型胶原蛋白免疫应答较弱，关节炎发生率低于10% （7）。

On the other hand, some CIA resistant mouse strains can produce arthritogenic antibodies, suggesting that CIA is not only

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restricted by MHC types. For example, INF-g 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 (8).

另一方面，CIA 抗性的小鼠也可能产生关节炎抗体。表明小鼠对 CIA 的敏感性不仅取决于MHC类型。 研究表明， CIA抗性的小鼠 C57BL/6, 129/Sv (H-2b), and Balb/c (H-2d)，当 INF-g 和 IL-10 基因敲除后，小鼠能产生关节炎自身抗体从而发生关节炎。这表明，对关节炎的敏感性也取决于不同细胞因子水平（8）。

A list of mouse strains commonly used for CIA and Collagen Antibody-Induced Arthritis (CAIA) are shown in Table 1.
列表一为用于CIA及抗胶原蛋白抗体诱导（CAIA）的关节炎动物模型研究的常用小鼠品系.

Table 1 - Mouse strains commonly used for CIA and CAIA

Mouse Strain	H-2 Type	CIA Susceptibility	Ref #	CAIA Susceptibility	Ref #	Note
DBA/1	q	High	2, 5, 6	High	13, 21	INFγ high
B10.Q	q	High	6	(High)		
B10.G	q	High	6	(High)		
NFR/N	q	High	38	(High)		
SWR	q	Resistant	17	Resistant		C5 deficient
B10.RIII	r	High	6	High	13	Low response: chick and human type II
B10	b	Low	10	(High)		* Need alternative immunization
C57BL/6	b	Low	10	Moderate - High	9, 18, 30	LPS low responder – * Need alternative immunization
C57BL/6 beige	b	Resistant	20	Resistant		PMN mutation
C57BL/6 x 129/Sv	b	Low	10	Moderate - High	30, 31	* Need alternative immunization
129/Sv	b	Resistant	10	High	27	
B10.D2/nSn	d	Resistant	20	High	20	
B10.D2/oSn	d	Resistant	20	Resistant	20	C5 deficient
Balb/c	d	Resistant		High	13	
Balb/c nu/nu	d	Resistant		Resistant	28	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	18	B & T cell deficient

Parenthesis-assumed, but not tested

*Develops arthritis by alternative immunization with CFA containing high concentrations of *M. tuberculosis*. *需要含高剂量结核杆菌的CFA诱导关节炎

D. Adjuvant
佐剂

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 antibody levels are necessary to activate complement, an essential step for inducing arthritis (9). 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) (10). However, because high concentrations of *M. tuberculosis* induce severe inflammation, please contact your institution's animal committee for guidance on choosing the appropriate CFA. The following is a list of adjuvants provided by Chondrex, Inc.

含有结核杆菌的完全佐剂对于诱发小鼠关节炎很重要。不同于大鼠，二型胶原加不完全佐剂（不含结核杆菌）无法诱导小鼠关节炎。产生抗体，包括IgG2a抗体对于激活补体及随后的关节炎的发生具有重要作用，取决于完全佐剂中结核杆菌的含量。最近，Campbell 用CFA（5mg/ml）在对CIA 抗性的小鼠中成功诱发高发病率的关节炎（50-70%），例如：C57BL/6, B10, and 129/Sv mice (H-2b) （10）。然而含高浓度结核杆菌的完全佐会诱发严重的炎症， 所以佐剂浓度（结核杆菌含量）取决于各研究单位动物管理委员会要求。以下是Chondrex,公司的佐剂列表。

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

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E. Collagen
胶原

Native, highly purified type II collagen prepared under a defined protocol should be used as deglycosylation of collagen will affect the arthritogenicity (11). Moreover, the failure to remove minor contaminants such as pepsin likely yields false positive reactions in T-cell proliferation assays (12). 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, Inc. offers a complete line of 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 only respond to chick type II collagen. 因为去糖基化的胶原会影响关节炎的产生（11），同时一些未去除的杂质比如：胃蛋白酶可能会导致

T 细胞刺激试验中产生假阳性（12），因此二型胶原应按规定高度纯化。如果按恰当的方法冻干的胶原-20℃避光保存的条件下，是非常稳定的。纯化的胶原溶于0.05M 浓度的醋酸 (Cat # 9075) 中，终浓度为2-4mg/ml，温和搅拌，4℃过夜。胶原溶液可在4℃保存一周，此后需在-20℃保存。Chondrex 公司提供较完整的不同免疫级别二型胶原. 比如，DBA/1 小鼠对鸡或者牛的二型胶原蛋白有较强的免疫反应，然后C57B/6 小鼠只对鸡的二型胶原蛋白有免疫反应。

Catalog #	Description
20011	Chick type II collagen,10mg 鸡II 型胶原蛋白
20012	Chick type II collagen, 5 ml x 2 mg/ml 鸡II 型胶原蛋白
20021	Bovine type II collagen, 10 mg 牛II 型胶原蛋白
20022	Bovine type II collagen, 5 ml x 2 mg/ml 牛II 型胶原蛋白
20031	Porcine type II collagen, 10mg 猪II 型胶原蛋白
20032	Porcine type II collagen, 5 ml x 2 mg/ml 猪II 型胶原蛋白
9075	0.05M Acetic Acid, 5 ml 醋酸

PROTOCOL TO INDUCE ARTHRITIS
关节炎诱发实验方案

A. Preparing the Emulsion
乳剂单位制备

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 effectively induce arthritis. In addition, sonication cleaves collagen into fragments which will be denatured at body temperature.

乳剂的质量对于诱发关节炎至关重要。乳剂的制备有多种方法。但是不推荐双注射器或超声波处理的方法。这些方法将导致乳剂不能够有效的、稳定的诱发关节炎。此外，超声处理方法，至少将胶原分成 2 个片段，这些小片段在正常体温下，很容易变性。

An electric homogenizer is highly recommended for preparing an emulsion:
推荐使用电动的高速搅拌器：

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). 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 to keep the emulsion cool during mixing, as heat will denature the collagen which will then fail to induce arthritis (Figure 3).

   用高速搅拌器 (图一)（带直径≤5mm 的刀片，图二a）搅拌完全佐剂CFA (或用于增强剂注射的不完全佐剂（IFA）)和胶原溶液 将注射器尖端用三通阀封闭 (图二b)。把注射器夹紧，固定在铁架台上，并冰浴 (图三)。最后一步很重要，因在搅拌过程中会发热，会引起蛋白变性，变性的蛋白不能够诱发关节炎（CIA）。

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Figure 1 – Homogenizer
图一 高速搅拌器

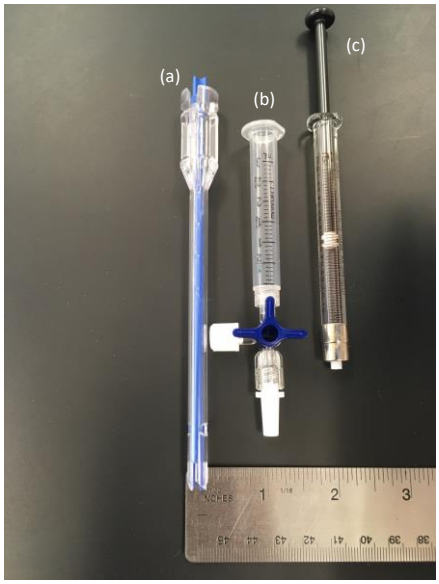

Figure 2 - Homogenizing blade - 0.5 cm diameter (a), Syringe with a 3-way stopcock (b), Hamilton glass syringe - 1 ml (c).
图二 (a) 搅拌器刀片-直径0.5cm, (b)带三通阀的注射器，（c）玻璃注射器

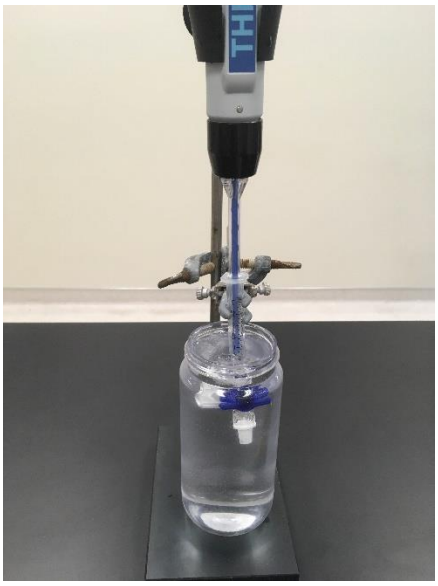

Figure 3 - A syringe sealed with a 3-way stopcock, clamped to a stand, and placed in an ice water bath.
图三 带三通阀的注射器固定在支架上，冰浴

2. Add one volume (maximum: 25% of the syringe volume) of CFA (IFA for booster injection) to the end of the syringe sealed with the 3-way stopcock. Then gradually add an equal volume of collagen solution (2 mg/ml in 0.05M acetic acid) dropwise while mixing at low speed (1000-3000 rpm).

   加 1 体积（最多不超过注射器的25%）完全佐剂（不完全佐剂在加强免疫时用到）到注射器，再缓慢加入等体积的胶原溶液（0.05M 醋酸溶解的的胶原溶液，终浓度为 2mg/ml），边低速搅拌（1000-3000rpm）边滴入胶原溶液。

   NOTE: To ensure a high-quality emulsion, the maximum emulsion volume should be no more than half of the syringe volume (50%). If more is needed, make several batches.

   注：为保证高质量的免疫乳剂，一次量乳剂体积应不得超过注射器体积的50%。如果需要多量生产，可重复以上步骤。

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 bath 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.

   继续高速混合（10,000 – 30,000rpm）2分钟。冷却5分钟，再重复高速混合和冷却步骤2-3次直到产生

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稳定的乳液。对于大体积乳剂制作（2-5ml）为了是乳液混合均匀，建议在混匀过程中上下移动搅拌刀片。

4. Replace the stopcock with a needle and 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 on the water's surface. If the emulsion dissipates onto the water surface, then the emulsion is not stable. Add a few drops of adjuvant, mix again, and retest.

   用针头替换三通阀，在装有冰水的烧杯里滴一滴乳剂，检测乳剂的稳定性。如果乳剂是稳定的，如图所示（图四），在水中是成团的，不散开。如果乳剂在水中散开，表明乳剂不稳定，再加几滴佐剂，重新混合再次进行测试。

Figure 4 - An intact, stable emulsion on the water's surface
图四 完整稳定的乳剂漂浮在水面

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.

   将乳剂转移到1ml的玻璃注射器 （图二C）.不建议采用塑料注射器，因为很难做到一个准确的注射剂量。

   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).

注：通过用力甩注射器将乳剂中的气泡赶出注射器（活塞部向下）。否则很难做到一个精确的注射剂量。

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

注：建议在乳剂制备好的一小时内完成注射。乳剂在使用之前存放在4°C。

B. Injection Site
免疫注射部位

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 2 cm from the base of the tail until the needle tip is 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 5). 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.

在鼠尾根部皮下注射 0.1ml乳剂(100µg胶原每只老鼠（图五）。例如：从尾根部 2cm 处插入针头（25号或者27号针头，长度5/8英尺，针插入皮下，针头沿斜向上与鼠尾方向平行插入，直到针尖插入位置距尾根部0.5cm。每次注射前充分擦拭针头，防止乳液的露出。如果要加强免疫，注射部位在尾根部 3cm 处，针尖插入皮下距鼠尾根部1.5cm处。加强免疫的注射部位应避开初始注射部位）。

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

注: Chondrex公司不建议使用背部皮下注射注射或腹腔注射胶原乳剂和佐剂，因为乳剂会引起腹腔和胸腔严重的炎症反应。

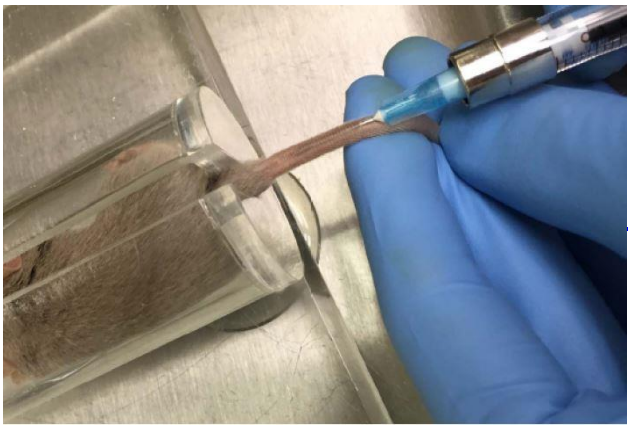

Figure 5 - Subcutaneous Immunization of Emulsion.

图五 皮下注射乳剂

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C. Immunization Schedule
免疫程序

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

根据小鼠品系和实验目的的不同，诱导高发病率和严重关节炎的方法也各有不同。

1. **Inducing arthritis by a single immunization without booster injection in HIGH RESPONDER strains**:
通过单次免疫（无需加强免疫）诱发关节炎（易感品系）

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

   注射胶原和完全弗式佐剂混合而成的含2mg/ml结合杆菌乳剂。对于易感品系小鼠DBA/1（H-2^q）和B10.RIII(H-2^r)注射乳剂后28-35天会引发关节炎。42-56天发病率达90-100%。关节炎严重程度高，临床评分达10-12分（最高16分）。

   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 (2) or (4).

   注：由于高浓度的结核杆菌会引起免疫部位严重的炎症反应，因此根据各研究机构对动物实验的要求，可选用方案2或方案4。

2. **Inducing arthritis with a booster injection in HIGH RESPONDER strains**:
通过加强免疫诱发关节炎（易感品系）

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

   注射胶原和完全佐剂混合而成的含0.5mg/ml结合杆菌乳.在初次注射后第21天，加强注射胶原和不完全佐剂（不含结核杆菌）混合而成的乳剂。尾部皮下注射0.1ml乳剂， 避开初次免疫部位。一般在初次免疫注射后28-35天引发关节炎。在第42-56天，发病率为80-100%，临床评分可达8-12分（最高16分）。

3. **Inducing arthritis with an alternative immunization protocol with high *M. tuberculosis* content adjuvant in LOW RESPONDER strains**:
用高剂量的结核杆菌完全佐剂加强免疫诱发关节炎（CIA抗性小鼠）

   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 collagen and CFA emulsion 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 of 2.5 mg/ml of *M. tuberculosis* on day 21. Arthritis will develop on days 28-35 after the first immunization. The maximum incidence of arthritis in these mice reaches approximately 50-70% on days 42-56 (10).

   对于一些低反应的小鼠，例如：B10 (H-2^b), C57BL/6 (H-2^b), and C57BL/6x129/Sv (H-2^b) ，采用以下方案可诱发关节炎。初次免疫，尾部皮下注射0.1ml胶原和完全佐剂混合而成的含2.5mg/ml结核杆菌的乳剂。在第21天加强免疫，再次注射0.1ml胶原和完全佐剂混合而成的含2.5mg/ml结核杆菌的乳剂。一般在初次免疫注射后28-35天引发关节炎。在第42-56天，发病率为50-70% （10）。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 no inflammation at the injection site as well as a dramatically shorter experimental period 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. Please visit www.chondrex.com for more information.

   注：该方案引起的炎症反应比较严重，根据各研究机构对动物实验的要求，可选用胶原抗体（CAIA）诱发的小鼠关节炎模型。Chondrex公司的单克隆抗体合剂（Arthrogen-CIA）和LPS能在CIA抗性的小鼠中诱发关节炎。相关信息请参考 www.chondrex.com。

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4. **Synchronizing onset of arthritis by LPS injection**:
通过LPS加强免疫

LPS has a synergistic effect in triggering arthritis with sub-arthritogenic doses of autoantibodies to type II collagen (13). Furthermore, severity and incidence in CIA can be increased by administering LPS (a B-cell mitogen), *Mycoplasma arthritidis* (a T cell mitogen) (MAM), and Staphylococcal enterotoxin B (SEB) (14-16). 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 collagen and CFA emulsion containing a final concentration of 0.5 mg/ml of *M. tuberculosis* according to protocol (2). 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.

LPS和体内二型胶原自身抗体水平对于诱发关节炎具有协同效应(13)。此外，在经典的CIA动物模型中，应用LPS(B细胞分裂素)或MAM(支原体产生的T细胞分裂素)或SEB(金黄色葡萄球菌产生的T 细胞分裂素)都会加强关节炎发病率和严重程度（14-16）。因此这些毒素不仅用于引发和加强关节炎水平，而且用于指定关节炎发生时间。

这个方案，需要注射0.1ml二型胶原和含0.5mg/ml的CFA混合乳剂（参考方案2）。在第25-28天或在在关节炎发生前3-5天腹腔注射LPS(25-50µg，溶解在生理盐水)。关节炎将在24-48小时发生，发病率90-100%。

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.

注：CFA免疫注射小鼠会在2-4周发生严重的免疫抑制。所以一些小鼠对LPS（50µg）注射非常敏感。Chondrex公司建议进行正式实验之前先对来自不同供应商的动物进行测试。

D. Onset of Arthritis
关节炎的发作

Clinically apparent arthritis with swollen joints appears 3-5 weeks with effective immunization. Onset and incidence of CIA depends on mouse strains and protocols.

有效的免疫注射后，临床上明显的关节肿胀症状会在3-5周出现。关节炎的发作和发病率取决于小鼠品系和实验方案。

EVALUATING ARTHRITIS
关节炎评估

A.Scoring
A.临床评分

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 CIA, adjuvant-induced arthritis, CAIA, and other inflammatory models. Chondrex, Inc. provides a scoring system (Table 2) and a supplemental flyer (please visit www.chondrex.com).

关节炎可通过临床评分来定性评估或用测厚仪来测量爪子厚度来评估。这些方法适用于 所有的关节炎模型比如经典的CIA，佐剂诱导的关节炎，单克隆抗体合剂和其他炎症模型。Chondrex公司提供一下系统（表二）

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

注：由于小鼠的爪子太小，因此无法像测量大鼠爪子体积那样通过浸没法测定小鼠爪子体积。

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Table 2 - Qualitative scoring system used to assess severity of paw inflammation.
表二 关节炎炎症程度的临床评分

Score 得分	Condition 发病情况
0	Normal 正常
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 轻度的、踝关节、腕关节发红、肿胀
2	Moderate redness and swelling of ankle or wrist 踝关节或腕关节中度发红肿胀
3	Severe redness and swelling of the entire paw including digits 爪子严重发红、肿胀，包括指端
4	Maximally inflamed limb with involvement of multiple joints 四肢最大程度发炎，包括多关节

B. Serum Analysis
B. 血清分析

High IgG autoantibody levels to mouse type II collagen are important for inducing arthritis (10, 17). More specifically, high levels of anti-type II collagen IgG2a and IgG2b subtype antibodies are required to activate complement, an essential step for inducing arthritis. Chondrex, Inc. provides mouse Anti-Collagen IgG and IgG subtype antibody ELISA kits to analyze the antibody levels. Please visit www.chondrex.com for more information.

高浓度自身二型胶原蛋白IgG抗体水平的产生是诱发关节炎的关键（10, 17）。二型胶原蛋白IgG2a 和 IgG2b抗体亚型对激活补体及随后的关节炎的发生至关重要。Chondrex公司提供小鼠胶原IgG及其各亚型抗体ELISA测试盒（具体信息参考www.chondrex.com）。

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