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K/BxN Serum Transfer Arthritis as a Model of Inflammatory Joint Pain

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Abstract

In this chapter, we describe the usage of this rheumatoid arthritis model to investigate pain-like behavior in mice, including the assessment of clinical changes and the time-dependent changes in nociceptive behavior during disease progresses.

Keywords

Rheumatoid arthritis; Persistent pain; Mice; Allodynia

1. Introduction

Chronic pain is a major health problem affecting approximately 20% of the population, resulting in markedly reduced quality of life for the individual as well as extensive costs for society. Approximately 1% of the population is diagnosed with rheumatoid arthritis (RA), a systemic autoimmune-mediated disease comprising synovial inflammation and matrix destruction. Swelling is particularly prominent in the small joints of the hands and feet. Pain is frequently the most egregious symptom reported and can persist after resolution of joint swelling with anti-inflammatory treatment. The inflammatory processes that result in rheumatoid arthritis are complex, with interactions among the cytokine network, autoantibodies, and the complement cascade. While the peripheral inflammation is an important component in generating pain in RA, the peripheral pathology cannot fully explain the amount of pain the arthritis patient experiences and facilitation of pain processing at the level of the spinal cord has been implicated (1). Despite the efficacy of new therapeutics (e.g., TNF and interleukin blockers) and treatment strategies (e.g., combination therapies), pain is still a significant problem. In a recent survey, more than 85% of RA patients described their RA as somewhat completely controlled, yet greater than 75% of them reported moderate to severe pain within the previous 2 months (2). Drug development in the area of chronic inflammatory pain has hitherto been insufficient. Thus, it is critical to increase our understanding for how chronic pain emerges during and subsequent to joint

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Factors contributing to variability in arthritis severity and potentially also hypersensitivity:

inflammation and how it is regulated in order to identify new targets and treatment strategies for pain relief. Here, we describe protocols for the usage of the K/BxN serum transfer arthritis (K/BxN) model, a well-described mouse model of inflammatory arthritis with similarities to rheumatoid arthritis, which has been characterized for studies of pain mechanisms and evaluation of analgesics (3). We believe that this model will add significantly to the repertoire of experimental “inflammatory pain” models. There is great interest in preclinical surrogate models for human inflammatory pain states. Models based on intraplantar injection irritants, such as carrageenan and formalin, have great popularity for the study of pain mechanisms and pharmacology. However, many of these models have a limited time frame (hours to days). Thus, changes in pain-related processes during chronic diseases, such as RA, may not be revealed and therefore there is a need for more chronic and disease-relevant models.

The KRN mice are transgenic for a T-cell receptor that is cognate for a peptide derived from bovine pancreatic RNase (4). A request for a material transfer agreement for the use of these mice can be made through the Institut de Génétique et de Biologie Moléculaire et Cellulaire (Strasbourg, France) and Drs. D. Mathis and C. Benoist (currently, Harvard Medical School, Boston, Massachusetts, USA). Crossing KRN mice on the C57Bl/6 background with nonobese diabetic (NOD) mice generates K/BxN mice. These mice develop severe destructive arthritis that resembles human rheumatoid arthritis in many respects. Serum transfer from the K/BxN mice reliably induces transient inflammatory arthritis in the joints of a wide range of mouse strains (4) utilizing autoantibodies against glucose-6-phosphate isomerase (5). This broad susceptibility distinguishes the K/BxN serum transfer model from other common models of RA, e.g., the collagen-induced arthritis model. As recipient mice receive the same quantity of autoantibodies at the time of injection, the K/BxN serum transfer model has a predictable onset of clinical signs of arthritis. The clinical profile has a severe inflammatory phase that reliably resolves as the antibodies are cleared and not replaced by B cells. While well-established for disease-mechanistic studies in the RA field, the K/BxN serum transfer arthritis model is new as an experimental model of inflammatory joint pain. Mice injected with K/BxN serum display robust and highly reproducible mechanical allodynia with an onset that correlates with joint and paw inflammation. Of importance, the mechanical hypersensitivity does not return to baseline concurrent with resolution of the joint swelling, but outlasts the inflammation by at least 2–3 weeks. Thus, this model provides an opportunity to study nociception not only during the ongoing joint inflammation, but also in the postinflammatory state.

2. Materials

2.1. Arthritis Induction and Scoring

1. Animals: Adult mice 25–30 g. This protocol is referring to male C57Bl/6 mice. K/BxN serum transfer is highly dependent upon the alternative complement pathway for arthritis development. Background strains have been screened for indications of clinical indices with Balb/c, C57Bl/10, PL, DBA/1, CBA, MRL/Mp, NZW, C3H/He, SJL, 120/Sv showing susceptibility to K/BxN serum transfer-induced arthritis, and DBA/2, FvB/N, NZB, and NOD showing no

induction of arthritis (6). If strains other than C57BL/6 are preferred, pilot studies and appropriate controls are suggested as different strains may show different susceptibility to K/BxN serum-mediated induction of hypersensitivity (and arthritis) (see Notes ¹ and ²).

2. K/BxN serum: Typically, 200 μ L per mouse total. See extensive protocols in refs. 7, 8 for the breeding and maintenance of the colony. Blood is collected either by complete exsanguination or by serial bleeding according to protocols as approved by local animal use committees. Blood is collected, briefly allowed to clot (10–20 min at room temperature), and then placed on ice. Samples are centrifuged for 5 min at 5,000 $\times g$ at 4°C and serum is pooled to produce maximal homogeneity of autoantibodies. Serum can be stored indefinitely at –70°C.
3. Control serum: Use serum from syngeneic mice and collect according to item 2. In this protocol, control serum is referring to serum collected from naïve C57BL/6.
4. 1-cc plastic syringe with 23-G needle for intraperitoneal (i.p) injection of serum.
5. Calipers.

2.2. Behavioral Testing

2.2.1. Von Frey

1. Von Frey stand with an elevated wire mesh surface with approximately 1/4" $\times$ 1/4"-square openings.
2. A clear plexiglass container placed on top of the wire mesh surface, in which to acclimate and test the mice. It must be large enough to allow the animals to turn around and tall enough that they cannot escape from it. A 3" $\times$ 3"-square box is sufficient.
3. Von Frey filaments (Stoelting. 0.04 g, 0.07 g, 0.16 g, 0.4 g, 0.6 g, 1.0 g, 2.0 g which correspond to 2.44 N, 2.83 N, 3.22 N, 3.61 N, 3.84 N, 4.08 N, 4.31 N).
4. Timer.

2.2.2. Thermal

1. Modified Hargreaves-type device: This includes a temperature- variable glass surface upon which the mice are placed and a triggerable, movable, focused heat source. Frequently, this heat source is attached to a mirror to allow for easy visualization of the heat source on the appropriate portion of the footpad. Here, we use a device from UARDG, Department of Anesthesiology, University of California, San Diego.

¹Strain selection: Genotypic and phenotypic studies by Ji et al. (6) outline the susceptibility of a variety of inbred strains and F2 generational crosses between responder/nonresponder strains. Pain-like behavior has not been assessed in most of these strains subsequent to induction of K/BxN serum transfer arthritis.

²Gender: It is recommended that investigators conduct pilot studies with appropriate controls to evaluate gender differences in pain-like behavior. A lack of sex-linked differences in clinical arthritis indices has been reported (6); however, gender-based pain-like behavioral differences have not been systematically explored.

2. Clear plexiglass containers that can be placed on top of the thermal testing device. These must be large enough to allow the animals to turn around and tall enough that they cannot escape from it. A 3" × 3"-square box is sufficient.
3. Timer.

2.2.3. Activity

1. Individual cages modified to allow insertion in the shorter end of an infrared motion detector which is shielded by a wire screen to supplement cage integrity.
2. Infrared motion detector (here, we use model SL-5407A) to establish positional changes caused by changes in mouse heat signatures.
3. Multichannel switching box to power detectors and interface via USB with computer running DigSigMon software. This software measures triggers (input) and data collection (output). The current configuration records an activity score of 0 (minimum) to 15 (maximum) per minute, tallied every minute continuously 24 h a day for multiple days. The room dedicated for activity testing is on timed 12-h light and 12-h dark cycle.
4. Mice are housed one per cage, up to 15 cages monitored in a dedicated room.

3. Methods

3.1. Arthritis Induction and Scoring

3.1.1. Prior to Serum Transfer, Determine the Ankle Thickness of Both Hind Limbs

1. Lightly scruff the mouse.
2. Place calipers over the thickest part of the ankle joint (malleoli) and lightly tighten until resistance. Read and record values. *Following arthritis induction, it is important to only tighten the calipers until they just touch the skin. The volume of edema present at the ankle from the arthritis can cause confound readings if it is compressed.*
3. Repeat every 1–3 days as desired.
4. Data can be reported as an increase (in mm) from baseline or as a percentage change from the baseline.

Ankle thickness and clinical scoring are usually maximal 4–8 days following induction of serum transfer. Panels a–c in Fig. 1 display three views of a control hind paw for comparison to panels d–f, which display views of a hind paw from a K/BxN serum transfer mouse on day 6 during maximal inflammation. These external indications of inflammation correspond to changes in the joint. In Fig. 1, panels g (day 6) and h (day 28), histology of ankle joints from control mice are contrasted to joints from K/BxN serum transfer arthritis mice, where the white arrows indicate inflammatory infiltrate in panel i (day 6) and black arrows indicate bone erosion in panel j (day 28). While clinical signs resolve, the ankle thickness frequently

remains slightly elevated from baseline due to a combination of aging and the remodeling of the joints induced by K/BxN serum transfer arthritis (Fig. 1j, k).

3.1.2. Prior to Serum Transfer, Determine Clinical Scores—There are multiple methods of scoring the clinical signs of arthritis in this model. Here, we describe two methods with the most important facet of scoring being consistency in the observer. From day 2 to 3 after K/BxN serum injection, the inflammation is quite obvious and with practice the scoring is highly reproducible. It is recommended though, that for more subtle investigations concerning alterations in clinical scoring from drug or genotype, deviations are conducted by an experienced, blinded investigator.

1. Lightly scruff the mouse.
2. Carefully examine and record clinical signs according to method 1 or method 2 (described below).
3. Repeat scoring every 1–3 days at the same interval as ankle thickness and other parameters are recorded.
4. Data are presented as a total score per time point.

Method 1: Data presented in this protocol are recorded using method 1. For each of the four limbs (maximum four points per limb, up to a combined total of 16), score points (1–4) according to the presence of the feature with the greatest point value.

1. point if there is only redness of the bottom of the footpad
2. points if there is visible thickening of the paw
3. points if the swelling of the ankle is sufficient to make the ankle equal to or greater in width than the mid footpad
4. points if there is swelling of at least one digit

Method 2: This is an extended scoring protocol that is commonly used in the research laboratory. The clinical score ranges from 1 to 15 for each paw, with a maximum score of 60 per mouse, based on the number of inflamed joints in each paw and inflammation being defined by swelling.

1. point per swollen digit, with a maximum of 5 per paw
2. points if swelling is observed on the dorsal side of the mid-paw
3. points if swelling of the ankle is observed

3.1.3. Arthritis Is Induced with Two 100 mL i.p. Injections of K/BxN Serum

1. Serum is stored frozen at -70°C . Thaw and lightly spin down serum prior to injection. Debris may still be present and should be avoided during injections.
2. On day 0, lightly scruff the mouse so that the ventrum is exposed and the head is pointing downward; this causes the freely move- able abdominal organs to move

toward the diaphragm, reducing the risk of accidentally puncturing internal organs.

3. Inject 100 μ L K/BxN serum or control serum i.p. Insert the 23-gauge needle into the abdominal cavity in the lower right quadrant of the mouse to avoid the cecum and urinary bladder. The needle should be directed toward the animal's head at an angle of 15–20° and inserted approximately 5 mm.
4. On day 2, inject 100 μ L K/BxN serum or control serum as described in Subheading 3.1.3.

Figure 1k shows a representative experiment in which the clinical score has been assessed with method 1. K/BxN serum-treated arthritic mice display maximal inflammation on day 6 and resolution of clinical signs by day 18. Figure 1 shows that the increase in clinical score correlates with an increased ankle thickness in K/BxN serum transfer arthritic mice. Due to swelling of the limbs, mice with K/BxN serum transfer arthritis have reduced gripping ability as compared to control mice. Food should, therefore, be placed on the bottom of the cage to ensure adequate nutrition and to prevent body weight loss. Long sipper tubes in the water bottles may be needed. Serum from naïve mice of the same strain as used in the study is the most appropriate control serum. see Note ³ regarding serum potency and variability.

3.2. Behavioral Testing

3.2.1. Von Frey

1. Habituation to testing device and baseline recordings. Mice are habituated to stay in the plexiglass containers for a minimum of an hour (depending upon strains; see Note ⁴) prior to testing. The experimenter must ensure that mice are calm and are no longer displaying exploratory behavior. We recommend to assess baseline thresholds with the same frequency as used in the experimental design, e.g., every third day, and to have a minimum of three baseline recordings prior to injection of K/BxN serum.
2. Multiple methods of testing mechanical hypersensitivity exist using von Frey filaments. We recommend the up–down method due to the reduced number of hair applications required.

The up–down method was originally developed for assessment of tactile allodynia in rats based upon Dixon (9). The method is described here only briefly.

Filaments are applied to the plantar surface between the toes for 5 s. A response results in clear and rapid lifting, shaking, or licking of the foot. Responses should be recorded from

³Serum potency: In this protocol, we recommend two 100- μ L injections for a robust induction of K/BxN serum transfer arthritis with a persistent pain-like state during the inflammatory phase as well as the postinflammatory phase to allow for maximal mechanical hypersensitivity and thus the testing of pharmacologic analgesics. To investigate factors suspected to enhance arthritic indices or mechanical hypersensitivity, it is recommended to reduce the injection volume to 50 μ L. As the spontaneously arthritic mice produce variable levels of anti- GPI antibodies over their lifetime, it is highly recommended for consistency to pool serum and use one pooled collection for the whole experimental series. Alternatively, the antibody titer level can be measured by ELISA. Protocols for antibody titer measurement are outlined in ref. 8. Maximal plasma anti- GPI antibody concentration is expected around 8–10 weeks of age (5). Serum can be obtained by either sacrifice of the animal at day 60 or by routine bleeding, as specified by local animal use committees.

⁴The length of acclimation period is strain dependent. For example, the length of time for acclimation among strains in our experience is C3H < C57Bl/6 < Balb/C.

one side of all mice (i.e., left) before testing on the right. In instances where the mouse begins walking, jumping, or exploring, the testing is discontinued and resumed after a period of 5–10 min.

In mice, testing begins with the middle hair (0.4 g) of the following set (0.04 g, 0.07 g, 0.16 g, 0.4 g, 0.6 g, 1.0 g, 2.0 g) and hairs are applied to collect 6 total responses. The first two responses should straddle the threshold (no withdrawal and then a withdrawal, or vice versa) and the following four are recorded according to the idea that (1) if no withdrawal response is recorded a stronger stimulus should be presented and (2) if a withdrawal response is recorded then a weaker stimulus is to be presented. Using the formula published by Chaplan in ref. 10, the 50% g threshold = $(10 [xf + kd])/10,000$, where xf = value (in log units) of the final von Frey hair used; k = tabular value (see Appendix in ref. 10 for the pattern of positive/negative responses); and d = mean difference (in log units) between stimuli.

Baseline 50% withdrawal values vary between strains but are expected to fall between 1.3 g and the maximum of 2.0 g. A description of relative differences in baseline mechanical hypersensitivity can be found in 11, with A/J, AKR/J, and BALB/c demonstrating high mechanical sensitivity at baseline and C57Bl/6, CBA/J, and SM/J the lowest baseline sensitivity. Data can be graphed as either (a) 50% threshold values or as (b) percent change from baseline.

Figure 2 shows a representative experiment in which the mechanical hypersensitivity was assessed with the up–down method in control and K/BxN serum transfer arthritic mice over a 28-day time period.

3.2.2. Thermal

1. Mice are acclimated in their plexiglass containers for a minimum of an hour (depending upon strains; see Note ⁴) prior to testing. The experimenter must ensure that mice are calm and are no longer displaying explorative behavior.
2. Here, we assess thermally evoked paw withdrawal responses using a Hargreaves-type testing device (12) (UARDG, Department of Anesthesiology, University of California, San Diego, 92103-0818).
 - a. Allow the glass surface to reach 30°C.
 - b. Initiate the thermal nociceptive stimulus (here, a focused projection bulb under the glass surface) coincident with starting the timer.
 - c. Terminate the stimulus and timer upon a brisk withdrawal of the paw.

Thermal latency is defined as the time required before withdrawal and is measured in seconds. Prior to experimental onset, change in the stimulus intensity can be achieved by altering the amperage of the bulb. Average baseline values typically fall within 8–12 s. To prevent thermal exposure damage, a cutoff time of 20 s is recommended. Figure 3 shows representative thermal sensitivity recordings spanning 28 days for control and K/BxN serum transfer arthritic mice. K/BxN serum transfer arthritic mice display hyposensitivity during day 3–6 post serum transfer as compared to control mice.

3.2.3. Activity—Measurement of mouse activity records a spontaneous index. A loss of activity may be secondary to disability, pain resulting from movement or a combination of both. In Fig. 4, the typical activity count during the dark cycle for control and K/BxN serum transfer arthritic mice is displayed. K/BxN serum transfer arthritic mice have a reduced dark cycle activity during day 2–8 following serum transfer.

1. Habituate mice individually in cages with motion detector attached. Recording should be taken from mice for at least 3–7 days prior to experimental onset to ensure accurate baselines.
2. Initiate arthritis as per instructions in Subheading 3.1. Care should be taken to handle mice as little as possible during activity monitoring experiments. If possible, injections should be given during the beginning of the light cycle (see Note ⁵).
3. Always dedicate one motion detector to monitor background room activity. It can be particularly useful to have a record of staff entering and exiting the room to correlate to any unexpected activity changes.
4. Using the DigSigMon software, monitor activity for desired length of time. We recommend at least 18–28 days with this model in order to see the full drop and resumption of activity from this model.
5. Data can be displayed in a wide variety of manner using manipulations available on the software. We suggest displaying total counts per 12-h period from the nocturnal cycle. Little change is noted during the light cycle as a result of K/BxN serum transfer arthritis. Because food and caging materials are changed during the light cycle, disruptions in counts are minimized with display of the nocturnal cycle. It is important to minimize the bedding and enrichment components which may block the IR beam. Alternative methods of data display and analysis include hourly binning of activity counts or the display of the full diurnal cycle.

References

1. Sokka T, Kautiainen H, Toloza S, Makinen H, Verstappen SM, Lund Hetland M, Naranjo A, Baecklund E, Herborn G, Rau R, Cazzato M, Gossec L, Skakic V, Gogus F, Sierakowski S, Bresnihan B, Taylor P, McClinton C, Pincus T. QUEST- RA: quantitative clinical assessment of patients with rheumatoid arthritis seen in standard rheumatology care in 15 countries. *Ann Rheum Dis.* 2007; 66:1491–1496. [PubMed: 17412740]
2. Taylor PC. The importance of the patients' experience of RA compared with clinical measures of disease activity. *Clin Exp Rheumatol.* 2010; 28:S28–31.
3. Christianson CA, Corr M, Firestein GS, Mobargha A, Yaksh TL, Svensson CI. Characterization of the acute and persistent pain state present in K/BxN serum transfer arthritis. *Pain.* 2010; 151:394–403. [PubMed: 20739123]
4. Kouskoff V, Korganow AS, Duchatelle V, Degott C, Benoist C, Mathis D. Organ-specific disease provoked by systemic autoimmunity. *Cell.* 1996; 87:811–822. [PubMed: 8945509]

⁵5. All experiments using activity as a measure of behavior resulting from the K/BxN serum transfer arthritis should incorporate as little handling of the mice as possible. Drugs via implanted pumps or available in the drinking water allow for the best separation between groups and avoid confounding effects due to handling.

5. Matsumoto I, Staub A, Benoist C, Mathis D. Arthritis provoked by linked T and B cell recognition of a glycolytic enzyme. *Science*. 1999; 286:1732–1735. [PubMed: 10576739]
6. Ji H, Gauguier D, Ohmura K, Gonzalez A, Duchatelle V, Danoy P, Garchon HJ, Degott C, Lathrop M, Benoist C, Mathis D. Genetic influences on the end-stage effector phase of arthritis. *J Exp Med*. 2001; 194:321–330. [PubMed: 11489951]
7. Monach P, Hattori K, Huang H, Hyatt E, Morse J, Nguyen L, Ortiz-Lopez A, Wu HJ, Mathis D, Benoist C. The K/BxN mouse model of inflammatory arthritis: theory and practice. *Methods Mol Med*. 2007; 136:269–282. [PubMed: 17983155]
8. Monach PA, Mathis D, Benoist C. The K/BxN arthritis model. *Curr Protoc Immunol*. 2008 Chapter 15, Unit 15 22.
9. Dixon WJ. Efficient analysis of experimental observations. *Annu Rev Pharmacol Toxicol*. 1980; 20:441–462. [PubMed: 7387124]
10. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods*. 1994; 53:55–63. [PubMed: 7990513]
11. Mogil JS, Wilson SG, Bon K, Lee SE, Chung K, Raber P, Pieper JO, Hain HS, Belknap JK, Hubert L, Elmer GI, Chung JM, Devor M. Heritability of nociception I: responses of 11 inbred mouse strains on 12 measures of nociception. *Pain*. 1999; 80:67–82. [PubMed: 10204719]
12. Dirig DM, Salami A, Rathbun ML, Ozaki GT, Yaksh TL. Characterization of variables defining hind- paw withdrawal latency evoked by radiant thermal stimuli. *J Neurosci Methods*. 1997; 76:183–191. [PubMed: 9350970]

Fig. 1.

Clinical and pathological signs following induction of K/BxN serum transfer arthritis.

Images of footpads (**a, b**) and joints (**c**) from control serum-treated mice are readily differentiable from images of footpads (**d, e**) and joints (**f**) from K/BxN serum transfer arthritis. The swelling in arthritic mice is sufficient to make the ankle wider than the midfoot region and to produce distinct redness and swelling of individual knuckles. Histopathology of the knee joints from arthritic and control mice on days 6 and 28 was sectioned and stained with hematoxylin and eosin. There is a prominent inflammatory infiltration on day 6 in the mice that received K/BxN sera (**i**; white arrow) and residual bony erosions on day 28 (**j**; black arrow), which are absent in control sections (**g, h**). Representative images are shown (original $\times 50$ magnification). Graphs display (**k**) arthritis clinical scores assessed for 28 days, demonstrating an increase in clinical signs of arthritis day 1–12 and (**l**) ankle thickness measured with calipers showing a significant ankle swelling in arthritic animals day 3–9. Each time point represents mean $\pm$ SEM ($n=9$ mice/group), * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ by Bonferroni post-test. Panels **g–l** have been reproduced from ref. 3 with permission from the International Association for the Study of Pain (IASP).

Fig. 2.

Characterization of mechanical hypersensitivity in mice subjected to K/BxN serum transfer arthritis. Graph displays 50% tactile thresholds (g) showing tactile allodynia day 2–28 (excluding day 12). Each time point represents mean±SEM ($n=9$ mice/group), * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ by Bonferroni post-test. Figure 2 has been reproduced from ref. 3 with permission from the International Association for the Study of Pain (IASP).

Fig. 3.

Characterization of thermal sensitivity induced by K/BxN serum transfer arthritis. Graph displays thermal thresholds (s) demonstrating that arthritic animals display thermal hypoalgesia day 3–6, with no other changes from baseline. Each time point represents mean $\pm$ SEM ($n=9$ mice/group), * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ by Bonferroni post-test. Figure 3 has been reproduced from ref. 3 with permission from the International Association for the Study of Pain (IASP).

Fig. 4. Activity monitoring in control mice and mice subjected to K/BxN serum transfer arthritis. *Graph* displays activity counts registered during the 12-h dark cycle. Arthritic mice display significantly reduced activity on days 3,4,5,7, and 8 following K/BxN serum transfer. Each time point represents mean $\pm$ SEM ($n=5-10$ mice/group), * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ by Bonferroni post-test.