The K/BxN Arthritis Model

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ABSTRACT

Mice expressing both the T cell receptor (TCR) transgene KRN and the MHC class II molecule A^{g7} (K/BxN mice) develop severe inflammatory arthritis, and serum from these mice causes a similar arthritis in a wide range of mouse strains, due to autoantibodies recognizing glucose-6-phosphate isomerase (GPI). K/BxN transgenic mice have been useful for investigating the development of autoimmunity, and the serum transfer model has been particularly valuable in eliciting mechanisms by which anti-GPI autoantibodies induce joint-specific inflammation. This unit describes detailed methods for the maintenance of a K/BxN colony, induction of arthritis by serum transfer, clinical evaluation of arthritis, and measurement of anti-GPI antibodies. *Curr. Protoc. Immunol.* 81:15.22.1-15.22.12. © 2008 by John Wiley & Sons, Inc.

Keywords: arthritis • rheumatoid arthritis • glucose-6-phosphate isomerase • GPI • anti-GPI • serum transfer • autoantibodies • mouse model • KRN • K/BxN

INTRODUCTION

Mice expressing the transgenic T cell receptor (TCR) KRN and the MHC class II allele A^{g7} (K/BxN mice) uniformly develop severe inflammatory arthritis, and serum from these mice reliably causes arthritis in a wide range of recipient strains (serum-transfer model) due to high levels of autoantibodies to the glycolytic enzyme glucose-6-phosphate isomerase (GPI). This model system has been useful for identifying genetic determinants of arthritis: first, by crossing the KRN and A^{g7} genes to other genetic backgrounds and second, and more powerfully, by transferring serum into a variety of gene-disrupted and inbred strains. The serum-transfer system allows for the rapid assessment of potential genetic determinants particular to the effector phase of arthritis, independent of determinants of the earlier step of breaking T cell tolerance. The following series of protocols is designed to allow any laboratory with general experience in mouse experimentation to set up a colony to produce arthritic K/BxN mice and conduct experiments using both the transgenic (see Strategic Planning) and serum-transfer (see Basic Protocols 2 and 3) models.

The maintenance of a colony to produce K/BxN mice requires two lines for breeders: (1) C57Bl/6 or B10.BR mice carrying the KRN transgene heterozygously and (2) NOD/Lt or other mice carrying the A^{g7} allele homozygously. KRN^+ mice are somewhat immunocompromised due to a limited diversity of TCR among T cells resulting from transgene expression, but they do not require special conditions for maintenance. Mice homozygous for KRN are viable but have reduced breeding performance, so depending on the facility, the colony overall will sometimes perform better using KRN heterozygotes, despite the inefficiencies of having to genotype breeders and of obtaining only 50% KRN^+ offspring. KRN^+ mice can be identified by flow cytometry (on the basis of a high number of Vβ6-positive T cells, see Basic Protocol 1) or by PCR of genomic DNA (see Alternate Protocol). The Support Protocol describes a method for measuring anti-GPI antibody titers.

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STRATEGIC PLANNING

In order to test the effect of a gene on spontaneous arthritis in K/BxN transgenic mice, it is necessary to first cross the KRN transgene and the H-2^{g7} locus separately onto the background with the gene to be tested (e.g., a disrupted gene denoted $X^{-/-}$), then breed these offspring to obtain K/BxN mice with the gene of interest, as outlined in Figure 15.22.1. The first cross generates $KRN^{+/-}X^{+/-}$ and H-$2^{g7/b}X^{+/-}$ animals. Since intercrossing these mice would produce a low yield (1/16) of the KRN^{+}H-$2^{g7/b}X^{-/-}$ mice of interest, it is generally preferable to include a second round of breeding to enrich for the relevant genes. Thus, $KRN^{+/-}X^{+/-}$ mice are intercrossed to generate

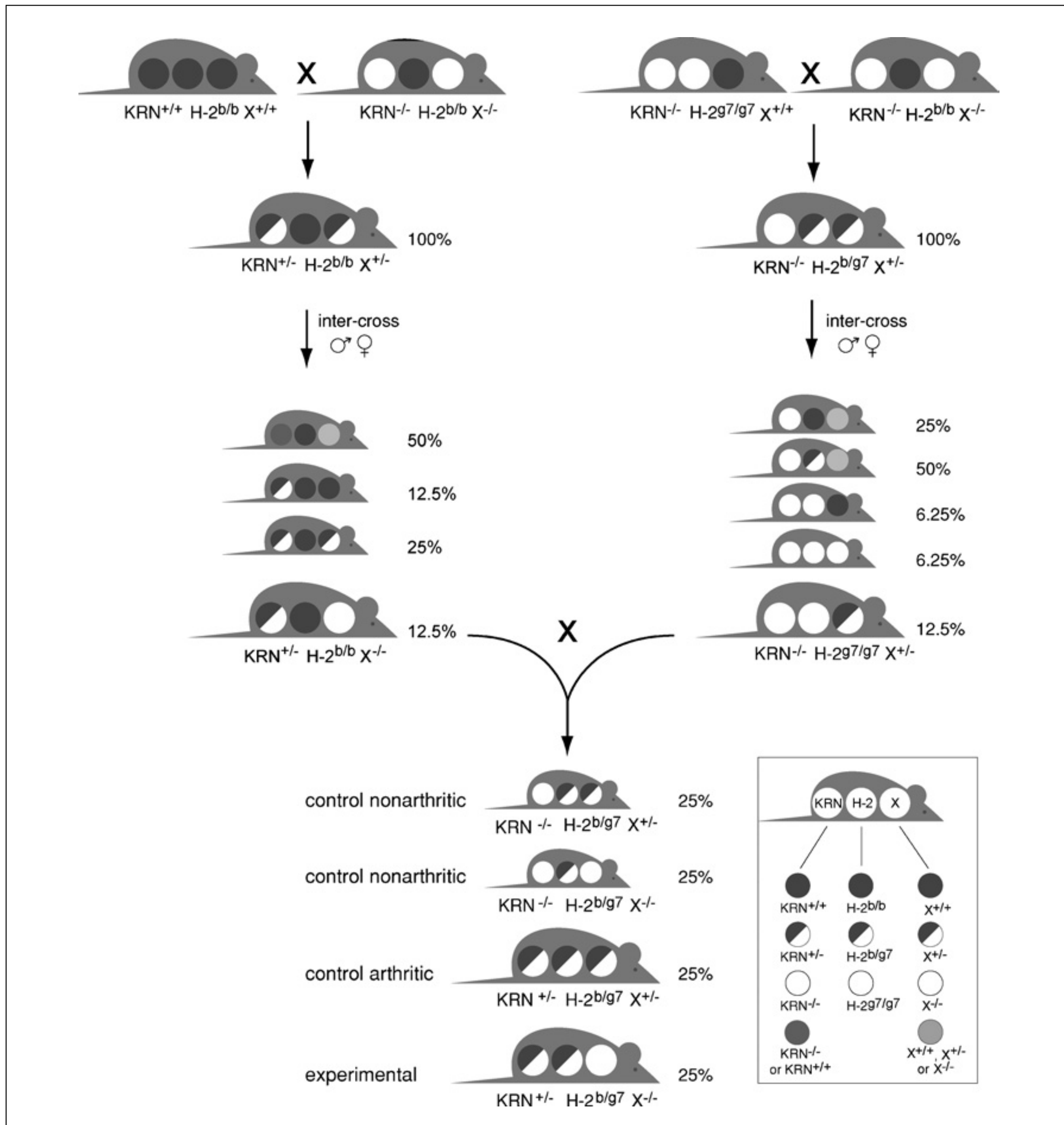

Figure 15.22.1 Crossing KRN and H-2^{g7} into other genetic backgrounds. A gene of interest X^{-} is first crossed separately to KRN (left side of figure) and H-2^{g7} (right side) so as to obtain experimental mice (equivalent to K/BxN on $X^{-/-}$ background) at a frequency of 25% in the third cross, along with arthritic K/BxN $X^{+/-}$ littermates for comparison. The breeding scheme will need to be modified if $X^{-/-}$ mice are unable to breed, resulting in 12.5% experimental mice in the final cross. Mice shown as smaller are those that are not used for subsequent breeding steps or experimentation. Gray circles indicate either + or − homozygotes of KRN or any genotype of X, in a setting in which any of these genotypes is not of interest for the next cross.

$KRN^{+/-}X^{-/-}$ offspring at a frequency of 1/8, and $H\text{-}2^{g7/b}X^{+/-}$ mice are intercrossed to generate $H\text{-}2^{g7/g7}X^{+/-}$ offspring at a frequency of 1/8. Finally, $KRN^{+/-}X^{-/-}$ mice are crossed to $H\text{-}2^{g7/g7}X^{+/-}$ mice in order to generate experimental $KRN^{+/-}H\text{-}2^{g7/b}X^{-/-}$ mice and control littermates $KRN^{+/-}H\text{-}2^{g7/b}X^{+/-}$, $KRN^{-/-}H\text{-}2^{g7/b}X^{-/-}$, and $KRN^{-/-}H\text{-}2^{g7/b}X^{+/-}$ at frequencies of 1/4 each.

As described above, $KRN^{+/+}X^{-/-}$ mice may be obtained in the first intercross at a frequency of 1/16 and could be used for the final cross in order to maximize the number of experimental mice, but breeding performance may be reduced, and all control littermates will have both KRN and $H\text{-}2^{g7}$ and thus develop arthritis. KRN^+ mice can be detected as in Basic Protocol 1 or 2. $H\text{-}2^{g7/b}$ and $H\text{-}2^{g7/g7}$ can be distinguished by flow cytometry of whole blood leukocytes using antibodies that distinguish A^{g7} from A^b (e.g., monoclonal antibodies 10.2.16 and Y-3P, respectively).

NOTE: All protocols using live animals must first be reviewed and approved by an Institutional Animal Care and Use Committee (IACUC) and must follow officially approved procedures for the care and use of laboratory animals.

SCREENING FOR KRN+ MICE BY FLOW CYTOMETRY

BASIC PROTOCOL 1

This protocol describes a flow cytometric method for confirming expression of the KRN transgene in putative KRN^+ mice. In KRN^+ mice, most $CD4^+$ cells are $V\beta 6^+$, compared to ~10% of $CD4^+$ cells in wild-type mice. Fluorescein-conjugated anti-mouse Vβ6 TCR antibody is used to assess the frequency of $V\beta 6^+$, $CD4^+$ T cells.

Materials

Male mice to be screened for KRN expression on the C57Bl/6 or B10.BR background (see Figure 15.22.1)
FACS wash buffer (see recipe)
Heparin
RBC lysis buffer (see recipe)
Fluorescein-conjugated anti-mouse Vβ6 TCR (e.g., Pharmingen 01364C)
PE-conjugated anti-mouse CD4 (e.g., Caltag RM2504-3)
1% (w/v) paraformaldehyde in phosphate-buffered saline (PBS; APPENDIX 2A)

Chamber to immobilize mouse
Heat lamp (optional)
Razor blade
1.5-ml plastic tubes
Flow cytometer capable of two-color analysis

1. From breedings of KRN^+ males and wild-type C57Bl/6 or B10.BR females, identify male offspring of agouti-gray coat color.

   *The KRN transgene is integrated near the Tyr locus and is closely linked to the agouti allele at that locus. Thus, most agouti-gray animals express KRN, and this phenotype can be used to select animals for genotyping, but should **not** be relied upon alone for identification of KRN^+ mice. In some colonies the KRN locus has been segregated away from the agouti allele, in which case KRN^+ mice are black.*

2. Immobilize a mouse and cut one of the lateral tail veins with a razor blade; collect 1 to 2 drops into a 1.5-ml tube containing 45 µl FACS wash buffer and 5 µl heparin. Mix and store on ice.

   Exposing the mouse to a heat lamp for 10 to 15 sec just prior to cutting the tail vein may be necessary to ensure that an adequate volume of blood is obtained.

3. Add 1 ml RBC lysis buffer to each tube. Incubate 4 to 10 min at room temp.

4. Centrifuge 5 min at 500× *g*, 4°C.

5. Discard the supernatant. Repeat the RBC lysis (steps 3 to 5) if the pellet is still mostly red.

6. Resuspend the pellet in 50 µl FACS wash buffer containing fluorescein-conjugated anti-mouse-Vβ6 and PE-conjugated anti-mouse CD4. Incubate 30 min on ice.

7. Add 1 ml FACS wash buffer and centrifuge 5 min at 500× *g*, 4°C.

8. Discard the supernatant, and resuspend the pellet in 100 to 200 µl of 1% paraformaldehyde in PBS.

9. Analyze cells by flow cytometry according to cytometer manufacturer's instructions.

   In KRN$^+$ mice, most CD4$^+$ cells are Vb6$^+$, compared with ~10% of CD4$^+$ cells in wild-type mice.

ALTERNATE PROTOCOL

SCREENING FOR KRN$^+$ MICE BY PCR

An alternative to flow cytometric screening of putative KRN$^+$ mice involves the use of PCR to confirm the genomic presence of the KRN transgene.

Materials

Male mice to be screened for KRN expression on the C57Bl/6 or B10.BR background (see Figure 15.22.1)
PK digestion buffer (see recipe)
20 mg/ml proteinase K (e.g., Sigma): prepare solution with water and store up to 1 year at −20°C
10× *Taq* polymerase buffer: 0.1 M Tris·Cl, pH 8.3 (*APPENDIX 2A*)/0.5 M KCl; store indefinitely at −20°C
50 mM magnesium chloride ($MgCl_2$)
5 M betaine: store up to 1 year at room temperature
10 mM PCR primers (5′ to 3′):
TCRαF: aggtccacagctccttctga
TCRαR: gtattggaaggggccagag
TCRβF: gggcaaaaactgaccttgaa
TCRβR: gagcctggttgtttgtggat
10 mM (each) dNTPs
5000 U/ml *Taq* polymerase (e.g., New England Biolabs)

Ear punch or scissors, sterile
50°C and 95°C water baths *or* heating block
Micropipettors and sterile filter tips
Tubes for thermal cycler
Thermal cycler

Additional reagents and equipment for performing agarose gel electrophoresis and staining and visualizing gels (*UNIT 10.4*)

1. Collect DNA from 3-mm (length) tail snips or 2-mm (diameter) ear punches by proteinase K digestion (or other standard protocol) as described below.

   Take stringent measures to avoid contamination between samples (e.g., carefully wipe scissors).

2. Submerge each tail snip or ear punch in 100 µl PK digestion buffer containing 100 µg/ml proteinase K added from 20 mg/ml stock just before use.

3. Incubate overnight at 50°C.

4. Incubate 10 min at 95°C to inactivate proteinase K. Store indefinitely at 4°C.

   Purity need not be very high because the PCR reaction is robust.

5. On ice and using filter tips, prepare the reaction mix for PCR amplification (per 25 µl reaction):

   2.5 µl of 10× *Taq* polymerase buffer (1× final)
   1.5 µl of 50 mM $MgCl_2$ (3 mM final)
   2.5 µl of 5 M betaine (1 M final)
   1.25 µl each of 10 mM primer (0.5 mM final)
   0.5 µl of 10 mM dNTPs (0.2 mM each final)
   0.1 µl *Taq* polymerase (0.5 U final)
   H_2O to total volume of 24 µl
   1 µl tail DNA sample from step 4 or no DNA (as a control).

6. Carry out PCR using the following amplification cycles:

Initial step:	5 min	95°C	(denaturation)
30 cycles:	1 min	95°C	(denaturation)
	1 min	60°C	(annealing)
	1 min	72°C	(extension)
Final extension:	5 min	72°C.	

7. Detect PCR products by agarose gel electrophoresis using a 1% agarose gel impregnated with ethidium bromide, visualized with UV illumination (see *UNIT 10.4*).

PRODUCTION OF ARTHRITIC K/BxN MICE AND ARTHRITOGENIC SERUM

BASIC PROTOCOL 2

KRN TCR transgenic mice are crossed with mice expressing the A^{g7} MHC allele. Production is optimized by using KRN males and NOD/Lt females since the latter produce large litters and the hybrid vigor of the BxNOD offspring is favorable to their general health. KRN males can be used for breeding from 7 weeks until about 10 months of age. NOD females are favorable, at least initially, because of their large litter sizes, but they succumb to diabetes between 12 and 25 weeks of age and must be replaced as breeders when excessive urination and/or weight loss become apparent. An alternative is the NOD.Ea16 transgenic line (available at *http://www.citdh.org*), which shares the entire genetic background of the NOD but is spared from diabetes by a protective MHC transgene; this transgene has no effect on arthritis in K/BxN progeny.

Materials

KRN^+ (on the C5B1/6 or B10.BR background) male mice, 7 to 10 months old (not commercially available; may request with MTA from authors)
NOD/Lt female mice, >6 weeks old (e.g., The Jackson Laboratory)

Breeding cages
1.5-ml plastic tubes and microcentrifuge

Additional reagents and equipment for identifying arthritic mice (Basic Protocol 3) and euthanizing (*UNIT 1.8*) and exsanguinating (*UNIT 1.7*) mice

1. Roughly calculate requirements for arthritogenic serum, considering the following:

   0.3 ml serum required per recipient mouse for a serum-transfer experiment
   0.4 ml serum per K/BxN mouse sacrificed
   4 to 5 K/BxN mice per litter
   Litters as frequently as every 3 weeks per female if the male remains in the cage after delivery but every 6 weeks if she is separated prior to delivery (as must occur if breeding trios are used as described below).

2. Set up breeding cages, each with one KRN^+ male and two NOD females.

3. Wean offspring and identify (see Basic Protocol 3) arthritic mice at 6 to 7 weeks of age, then maintain until 9 weeks of age (when the titers of anti-GPI antibodies are maximal).

   Severely arthritic mice should be easily identifiable even by an untrained observer. Mild arthritis is rare, and such mice should not be used to obtain serum for transfer.

4. Sacrifice (see UNIT 1.8) and completely exsanguinate mice by an accepted protocol (e.g., cardiac puncture on anesthetized mice; see UNIT 1.7). Allow blood to clot for 30 to 60 min at room temperature.

5. Prepare serum by centrifuging 5 min at 500× *g*, 4°C.

6. Discard the pellets (containing cells and debris). Pool serum obtained on a given day, pipet 1-ml aliquots into microcentrifuge tubes, and store indefinitely at −80°C until use.

   Ideally, each tube would be thawed only once prior to use, but in order to conserve serum it is often advisable to refreeze a tube once.

BASIC PROTOCOL 3

INDUCTION AND EVALUATION OF ARTHRITIS BY SERUM TRANSFER

The K/BxN serum transfer model has become widely used for two reasons: (1) it allows focus on mechanisms of the effector phase of inflammatory arthritis, after autoantibodies have been produced, and (2) it can be used on a wide range of strain backgrounds since the KRN TCR and A^{g7} MHC allele are not required for this model. The clinical evaluation of arthritis as described below can also be used for experiments involving the K/BxN transgenic model, in which genes of interest are introduced into the strain backgrounds used to produce these offspring (see Strategic Planning).

Materials

K/BxN serum (Basic Protocol 2)
5- to 7-week-old male mice or 6-week-old female mice (see Strategic Planning)
0.5- to 1-ml syringe and 25- to 27-G needle
Precision caliper (e.g., Kafer dial thickness gauge with flat anvils, Long Island Indicator)

1. Starting just before the first injection of serum, measure one or both ankles of mice:

   a. Immobilize the mouse in one hand.
   b. Measure the ankle at the widest point (the malleoli). Read the meter as soon as both sides of the caliper touch the ankle.
   c. Also examine all four paws to make sure that no swelling is apparent prior to arthritis induction.

   Measurement of one ankle is sufficient for many experiments, but both are measured for critical experiments in which maximum robustness of data is desired.

   The ankle thickness of a normal young-adult mouse is usually 2.7 to 3.0 mm. Trauma can cause swelling that can confound experiments; fighting among male mice is a common cause. If evaluating the spontaneous arthritis that arises in K/BxN mice, begin measurements and clinical assessment at 22 days of age, i.e., before disease onset.

2. Inject 0.2 ml serum intraperitoneally into each mouse (see UNIT 1.6).

3. On day 1, repeat ankle measurements, and on day 2, repeat measurements and serum injection.

4. In addition, assess all four paws subjectively for inflammation, on a scale of 0 to 3, where:

   1 indicates mild swelling of the ankle insufficient to reverse the normal V shape of the foot (i.e., the foot at the base of the toes remains wider than the ankle and hindfoot)
   2 indicates swelling sufficient to make the ankle and midfoot approximately equal in thickness to the forefoot
   3 indicates reversal of the normal V shape of the foot.

   Apply analogous guidelines for evaluation of the wrist, with swelling of only one or two digits but not the ankle or wrist scored as 1.

   Once inflammation is present, it is particularly important to take the measurement as soon as the gauge contacts tissue on both sides, since the inflamed tissue is soft and easily compressed. In the event that the first attempt at measurement fails, wait 5 min before attempting to measure again.

5. Repeat measurements and clinical scoring every 2 to 3 days.

   Arthritis severity is usually maximal 7 to 14 days after the first serum injection and declines slowly over the subsequent 2 to 3 weeks. Although inflammation resolves, ankle thickness usually remains greater than baseline, sometimes by up to 0.5 mm, due to new bone formation.

6. *Optional:* At the conclusion of the experiment, collecting serum and ankle tissue.

   If some mice do not develop severe arthritis, measurement of anti-GPI antibody titers (see Support Protocol) in the recipient mice can provide reassurance that they were successfully injected with K/BxN serum.

   Ankle tissue can be processed for frozen or paraffin sectioning (see Commentary).

7. Plot data both as clinical index (0 to 12 based on 0 to 3 scores in each of 4 paws) and as ankle thickening, by subtracting the baseline ankle thickness of each paw from its subsequent measurements.

SUPPORT PROTOCOL

MEASUREMENT OF ANTI-GPI ANTIBODY TITERS

Recombinant glucose-6-phosphate isomerase (GPI) can be expressed at high levels (100 mg per liter of culture is not unusual) as a fusion protein with GST or with a His-tag, using standard protocols. We have generally used the GST fusion for ELISA as described below (Matsumoto et al., 1999). This ELISA assay is useful for measuring the development of autoantibodies over time in K/BxN mice, confirming the presence of high-titer anti-GPI antibodies in a K/BxN colony, verifying the activity of anti-GPI IgG purified from K/BxN serum, and verifying successful injection of serum in serum-transfer experiments. Mouse sera do not react to the GST moiety nor to the BSA used in the blocking buffer; this is not true of many human sera, so BSA should be included in the Ab dilution buffer when using human sera to keep any BSA-binding antibodies in solution.

Materials

E. coli containing plasmid expressing mouse GPI as a fusion protein (e.g., mouse GPI in pGEX-4T-3; Amersham/Pharmacia)
2× YT medium (see recipe)
Isopropyl-β-D-thiogalactopyranoside (IPTG)
Phosphate-buffered saline (PBS; *APPENDIX 2A*), 4°C
Triton X-100
50% slurry of glutathione Sepharose 4B (Amersham/Pharmacia) in PBS (*APPENDIX 2A*)
Elution buffer (0.154 g reduced glutathione per 50 ml of 50 mM Tris·Cl, pH 8.0)
Blocking buffer: PBS with 1% bovine serum albumin (BSA)

Mouse sera to be tested (see Basic Protocol 2)
Alkaline phosphatase-conjugated goat-anti-mouse-IgG: e.g., $F(ab')_2$ fragments (Jackson Immunoresearch)
Phosphatase substrate (e.g., pellets from Sigma)
Phosphatase buffer (e.g., 8% diethanolamine/2.4 mM $MgCl_2$, pH 9.8)

Shaking incubators, 30°C and 37°C
UV/visible light spectrophotometer with appropriate cells for measuring bacterial cultures and protein concentrations
100- to 500-ml centrifuge bottles and 15- to 50-ml centrifuge tubes
Refrigerated centrifuge equipped with regular rotors and rotor adapted for 96-well plates
Probe sonicator
Test tube rotator
Dialysis tubing, 10,000 mol. wt. cutoff (e.g., Slide-a-Lyzer, Pierce)
Flat-bottom 96-well plates
Micropipettors including multichannel pipettor
Microplate spectrophotometer

Prepare recombinant mouse GPI lysate

1. Prepare a 10 ml overnight culture of *E. coli* containing the expression plasmid.

   The resulting fusion protein has GST at the N-terminus.

2. Dilute the overnight culture into 1 liter of 2× YT medium and incubate at 30°C, with shaking, until the A_{600} is between 0.5 and 2.
3. Add IPTG to 0.1 mM and continue incubating for an additional 4 hr.
4. Centrifuge the culture 10 min at 3000 × *g*, 4°C.
5. Discard the supernatant and keep the pellet on ice. Resuspend the pellet in 50 ml cold PBS.
6. Sonicate in short bursts until partial clearing is apparent.
7. Add Triton X-100 to 1% and incubate 30 min on ice.
8. Centrifuge 10 min at 12,000 × *g*, 4°C.
9. Discard the pellet and save the supernatant (lysate).

Purify GPI from lysate

10. Equilibrate 2 ml of a 50% slurry of glutathione Sepharose with PBS.
11. Combine the lysate and glutathione Sepharose beads and mix 30 min at room temperature, with rotation.
12. Wash the beads three times with PBS (10 ml/wash), collecting the beads each time by low-speed centrifugation (1 min at 500 × *g*, 4°C).

    Alternatively, the beads may be placed into a column for washing and/or elution.

13. Add 1 ml elution buffer to the 1 ml of packed beads and incubate 10 min at room temperature.
14. Centrifuge 5 min, at 500 × *g*, 4°C.
15. Repeat the elution twice, pool the three supernatants, and dialyze against three changes (24 hr each) of 1000 ml PBS at 4°C.
16. Determine the protein concentration by UV spectrophotometry (assuming an approximate A_{280} of 1.4 for a 1 mg/ml solution).

17. Store in small aliquots indefinitely at −20°C or colder.

    Enzymatic activity of GPI is unstable at −20°C (C. Jeffrey, pers. comm.), but the fusion protein continues to perform reliably in ELISA.

Perform anti-GPI ELISA

18. Thaw an aliquot of the recombinant GPI and dilute to 5 µg/ml in PBS.

19. Add 100 µl to each well of a flat-bottom 96-well plate suitable for ELISA.

20. Allow the protein to bind to the plate overnight (or longer) at 4°C.

21. Discard the coating solution and wash once with ∼200 µl of PBS per well.

22. Add 150 µl/well blocking buffer and incubate 30 min at room temperature.

23. Wash once with ∼200 µl of PBS per well.

24. Add dilutions of sera to be tested, in PBS or PBS/BSA, incubate 60 min at RT.

    The linear range of anti-GPI activity in K/BxN serum is typically seen between dilutions 1:3000 and 1:1,000,000. The activity in sera 2 weeks after transfer of K/BxN serum for induction of arthritis is typically 10- to 30-fold lower, so the starting dilution used is 1:100 or 1:300.

    Mouse sera do not react to the GST moiety nor to the BSA used in the blocking buffer; this is not true of many human sera, so BSA should be included in the antibody dilution buffer when using human sera.

25. Wash three times with ∼200 µl of PBS per well.

26. Add alkaline-phosphatase-labeled anti-mouse-IgG, typically diluted ∼1:2000 in PBS or PBS/1% BSA. Incubate 60 min at room temperature.

27. Wash three times with ∼200 µl of PBS per well.

28. Add phosphatase substrate in appropriate buffer. Incubate at room temperature, protected from light.

29. Read absorbances using a microplate spectrophotometer.

    With Sigma phosphatase pellets in diethanolamine buffer, plates are read at 405 nm after 5 to 10 min incubation.

REAGENTS AND SOLUTIONS

Use deionized, distilled water in all recipes and protocol steps. For common stock solutions, see **APPENDIX 2A***; for suppliers, see* **APPENDIX 5***.*

FACS wash buffer

Phosphate-buffered saline (PBS; *APPENDIX 2A*)
30 mM HEPES ph 7.4
3% heat-inactivated horse serum
0.1% sodium azide
Store up to 1 year at 4°C

PK digestion buffer

10 mM Tris·Cl, pH 8.3 (*APPENDIX 2A*)
50 mM KCl
0.01 mg/ml gelatin
0.045% NP-40
0.045% Tween 20

continued

Prepare the Tris/KCl/gelatin solution and store indefinitely at −20°C. Add NP-40 and Tween 20 before use from 10× stocks (prepared in water and stored up to 1 year at room temperature).

RBC lysis buffer

10 mM HEPES pH 7.3
0.15 M NaCl
0.1 mM EDTA
Store up to 1 year at room temperature

YT medium, 2×

16 g Bacto tryptone (Difco)
10 g Bacto yeast extract (Difco)
5 g NaCl
Sterile H_2O to 1000 ml
Adjust pH to 7.0
Autoclave 25 min
Store indefinitely at room temperature

COMMENTARY

Background Information

Among the many mouse models of inflammatory arthritis (see Monach et al., 2004), the K/BxN model has the following distinguishing combination of features: (1) spontaneous disease with no requirement for immunization or adjuvant (Kouskoff et al., 1996); (2) incidence of disease dependent only upon loss of tolerance in a particular TCR-MHC interaction, without mutation of genes more broadly affecting immune homeostasis; (3) a known target antigen, glucose-6-phosphate isomerase (Matsumoto et al., 1999); (4) ability to transfer disease to a wide range of recipient strains using serum containing the pathogenic autoantibodies (Korganow et al., 1999), without a need for purifying or concentrating these antibodies; (5) robust development of arthritis in 100% of transgenic animals and nearly 100% of wild-type mice that have received K/BxN serum; and (6) absence of significant inflammation in other organs.

The relative ease and reproducibility of the serum transfer model has led to a rapid delineation of cell types and immune mediators required for the “effector phase” inflammatory response that follows autoantibody production. These include mast cells (Lee et al., 2002) and neutrophils (Wipke and Allen, 2001), but not lymphocytes (Korganow et al., 1999) or synovial-resident macrophages (Bruhns et al., 2003); the alternative pathway of complement and the C5a receptor, but not the classical pathway, the membrane attack complex, or the complement receptors CR1, 2, and 3 (Ji et al., 2002a); the stimulatory Fc receptor FcγRIII (Ji et al., 2002a); the inflammatory mediators IL-1, TNF (Ji et al., 2002b) and β-leukotrienes (Chen et al., 2006; Kim et al., 2006); and the adhesion molecule LFA-1 (CD11a/CD18), but not Mac-1 (CD11b/CD18; Watts et al., 2005). The discovery that inflammatory arthritis could be the sole consequence of autoantibodies directed against a ubiquitous antigen led to studies that have identified a unique responsiveness of the synovial vasculature to immune complexes and vasogenic amines (Binstadt et al., 2006; Wipke et al., 2004) and helped to reinvigorate the concept that B cells and autoantibodies may play an important pathogenic role in rheumatoid arthritis (RA; Edwards et al., 2004).

Among the many other mouse models of RA, collagen-induced arthritis (CIA; *UNIT 15.5*) has been the most informative thus far. Immunization of susceptible mouse strains with type II (cartilage-specific) collagen in adjuvant leads to arthritis in ∼50% of mice several weeks later (Courtenay et al., 1980). Genetic determinants of the induction of immunity to type II collagen have been readily determined, but the inability to separate the immunization and effector phases initially made the roles of required mediators less clear. The identification of an arthritogenic mixture of monoclonal antibodies (MAbs) to type II collagen (Kagari et al., 2002), now commercially available, has increased the power of the CIA model to delineate disease mechanisms, analogously to the development of the K/BxN serum

transfer model. Thus far, findings in these two models have been quite similar (Monach et al., 2004).

Arthritis can also be induced using a mixture of MAbs directed against different epitopes on GPI (Maccioni et al., 2002). However, since the arthritis elicited by a mixture of three MAbs is far less robust than that elicited by K/BxN serum, and, since even a mixture of seven MAbs remains less robust, we continue to use serum rather than MAbs.

Critical Parameters

Probably the most important factor in conducting successful experiments using K/BxN mice and the serum transfer model is the clinical assessment of arthritis. Both the qualitative (grading of severity in each paw on a scale of 0 to 3) and quantitative (ankle measurement with a caliper) assessment of arthritis severity necessarily improve with experience, but that task is made easier by the fact that in most strains, arthritis ~4 days after serum transfer is so severe as to be unambiguous even to the untrained observer.

Experiments testing factors with subtle influences on arthritis severity would best be done after the researcher is comfortable with these assessments, and preferably in a blinded fashion. In addition, when subtle factors are being tested, consideration of effects of strain-associated genes (Ji et al., 2001; Ohmura et al., 2005) must be kept in mind, and extensive back-crossing of recipient strains may be required. When attempting to assess factors that are expected to increase rather than decrease severity of arthritis, a lower dose of serum than that listed in Basic Protocol 3 should be used, e.g., 50 to 100 μl rather than 150 μl for each of the two injections for recipient on the C57Bl/6 background.

Since one described mechanism of resistance to serum-transferred arthritis is a marked reduction in the half-life of circulating anti-GPI antibodies (Akilesh et al., 2004), it is wise, after identification of a novel factor associated with resistance, to measure anti-GPI titers in recipient mice 1 to 3 weeks after injection in order to address this possibility.

Troubleshooting

As above, the use of male breeders homozygous for the KRN transgene ensures that all the offspring will be arthritic and can be used for serum collection. However, the better breeding performance of heterozygous $KRN^{+/-}$ males may in some cases make it more economical to use these as breeders and be resigned to producing only 50% arthritic offspring.

The breeding scheme described in Strategic Planning assumes that mice homozygous for the mutant/disrupted/transgenic gene of interest will be viable breeders. If this is not the case, then the cross that produces experimental $KRN^{+/-}$ $H\text{-}2^{g7/b}X^{-/-}$ mice will by necessity be made using $X^{+/-}$ breeders, with a predictable 2-fold reduction in frequency of the desired offspring.

In general, individual lots of K/BxN serum are reliably arthritogenic and do not need to be confirmed as such prior to use in experiments. Rarely, however, a serum-producing colony may drift to produce less-arthritogenic serum over time. In such a case, sera from individual K/BxN mice should be tested for anti-GPI titers (see Support Protocol) and pedigrees evaluated to see whether particular breeding pairs have produced multiple low-titer offspring and should be removed from the colony.

Anticipated Results

When evaluating genes and treatments with profound effects on arthritis incidence and severity (characteristic of most of the published findings using this model), convincing and statistically-significant results can often be generated using only four to five mice per group. More subtle influences will necessarily require larger groups.

In addition to clinical assessment of arthritis, researchers may wish to confirm the presence or absence of inflammation, cartilage destruction, bone erosion, deposition of IgG and complement, etc. Although we have had success staining paraffin sections for IgG, staining for C3 deposition has only been successful with the use of frozen sections cut from non-decalcified bone, which requires specialized procedures to minimize fragmentation of the tissue (Ji et al., 2002a; Lee et al., 2002). Paraffin sections can be used for the other purposes listed, but the tissue must be decalcified prior to sectioning. This can be done either rapidly using formic or nitric acid (Kouskoff et al., 1996), over 2 days with Kristensen's solution (a mixture of 20% 8 N formic acid and 80% 1 N sodium formate), or over 2 weeks with 0.375 M EDTA (pH 7.5) at 4°C (4 ml/ankle, changed twice per week). The gentler the decalcification method used, the more likely that structures detectable by immunohistochemistry will be preserved.

Time Considerations

Screening of 10 to 20 mice for KRN by flow cytometry can be performed in 3 to 4 hr. Screening by PCR takes longer due to the initial step of preparing tail DNA (e.g., incubation

overnight with proteinase K), but it requires less labor. Serum can be collected from a large number of mice in 1 to 2 hr.

A mouse can be assessed clinically for arthritis and have ankle measurements performed and recorded within 1 to 2 min by an experienced investigator.

Starting with KRN^+ and $Ag7^+$ breeders, serum production can begin with the first litters, i.e., in about 3 months. Production of K/BxN mice bearing other genes of interest requires three generations (see Strategic Planning) and thus at least 9 months.

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