FINAL SCRIPT: APPROVED FOR FILMING

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Title: An Adoptive Transfer Model of Rheumatoid Arthritis in Mice

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **Yes**

Current Protocol Length
Number of Steps: 20
Number of Shots: 45

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Zijun Ma:** The scope of this research is to establish a rapid and stable animal model of Rheumatoid Arthritis for study of pathogenesis and molecular mechanism.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.3*

What are the current experimental challenges?

1.2. **Zijun Ma:** Current rheumatoid arthritis animal models face limitations in experimental practicality, including prolonged induction timelines, suboptimal cost-effectiveness, and inconsistent disease phenotype reproducibility.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.2*

What significant findings have you established in your field?

1.3. **Zijun Ma:** Here we developed a novel rheumatoid arthritis model by adoptively transferring SKG mouse CD4+ T cells into wild-type C57BL/6 mice, achieving a high incidence with 100% success within 14 days which is a lot faster than conventional methods.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.1*

What advantage does your protocol offer compared to other techniques?

1.4. **Zijun Ma:** This cost-effective, highly reproducible system uniquely enables precise immune mechanism analysis and therapy testing.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Animal Ethics Committee at Tongji Medical College

Protocol

2. Isolation and Purification of CD4⁺ T cells from SKG mice

Demonstrator: Zijun Ma

2.1. To begin, immerse euthanized SKG *(S-K-G)* mice in 75% alcohol for 5 minutes to disinfect **[1-TXT]**. Locate the spleen in the abdominal cavity and the lymph nodes in the inguinal and popliteal regions **[2]**.

2.1.1. WIDE: Talent submerging mice in a beaker filled with 75% alcohol. **TXT: Euthanasia: CO_2 asphyxiation**

2.1.2. Talent locating the spleen in the abdominal cavity and identifying the inguinal and popliteal lymph nodes.

2.2. Use sterile forceps and scissors to carefully dissect the spleen and lymph nodes **[1]** and immediately transfer them into prechilled PBS **[2]**.

2.2.1. Talent dissecting the tissues.

2.2.2. Talent placing them into tubes containing prechilled phosphate-buffered saline.

2.3. Place the spleen and lymph nodes in separate Petri dishes **[1]**. Then press the tissues through a 70-micrometer cell strainer using the plunger of a sterile syringe **[2]**, while gradually adding 10 to 12 milliliters of prechilled PBS to form a uniform cell suspension **[3]**.

2.3.1. Talent placing spleen and lymph nodes in separate Petri dishes.

2.3.2. Talent using syringe plunger to press tissues through cell strainer.

2.3.3. Shot of phosphate-buffered saline being added to the strainer.

2.4. Pass the cell suspension through the same cell strainer into a 15-milliliter centrifuge tube **[1]**. Then centrifuge the tube at 300 *g* for 7 minutes at 4 degrees Celsius **[2]**. Discard the supernatant and retain the cell pellet **[3]**.

2.4.1. Talent transferring cell suspension through the same strainer into a 15 mL tube.

2.4.2. Talent placing the tube into a centrifuge and starting the spin.

2.4.3. Talent removing tube from centrifuge and discarding the supernatant.

2.5. Adjust the cell concentration to 10^8 cells per milliliter using an appropriate amount of buffer provided in the CD4⁺ T cell *(C-D-Four-Plus-T-Cell)* isolation kit **[1]**. Then mix 10 microliters of cell suspension with trypan blue to assess cell viability **[2-TXT]**.

2.5.1. Talent adding an appropriate amount of buffer to the cell pellet and mixing gently.

2.5.2. Talent transferring 10µl cell suspension into a vial and mixing with trypan blue. **TXT: Count cells and confirm ≥90% cell viability.**

2.6. Now transfer 100 microliters of the cell suspension into a new tube **[1]**. Pipette 10 microliters of Biotin-Antibody-Cocktail into the tube **[2]**, mix thoroughly, and incubate on ice for 15 minutes **[3]**.

2.6.1. Talent transferring 100 microliters of cell suspension into a new microcentrifuge tube.

2.6.2. Talent adding Biotin-Antibody-Cocktail into the cell suspension.

2.6.3. Talent mixing by pipetting and placing the tube on ice.

2.7. Resuspend the beads by vortexing at maximum speed **[1]**. Add 10 microliters of the Streptavidin bead suspension to the tube, mix well, and incubate on ice for 15 minutes **[2]**.

2.7.1. Talent vortexing bead vial vigorously.

2.7.2. Talent adding Streptavidin beads to tube, mixing, and placing back on ice.

2.8. Next, add 2.5 milliliters of the kit buffer to the tube **[1]** and place it on a magnetic separation rack for 5 minutes **[2]**.

2.8.1. Talent adding buffer to the tube.

2.8.2. Talent placing it into the magnetic separation rack.

2.9. Carefully pour the liquid, containing the target cells, into a new sterile tube **[1]**. Centrifuge the tube at 300 *g* for 5 minutes at 4 degrees Celsius **[2]**. Then discard the supernatant and retain the cell pellet **[3]**.

2.9.1. Talent decanting liquid into a fresh tube.

2.9.2. Talent placing the tube in the centrifuge and starting spin.

2.9.3. Talent removing tube and discarding the supernatant.

2.10. Now add enough sterile PBS to adjust the cell concentration to 2 million cells per milliliter **[1]** and keep the suspension on ice for later use **[2]**.

2.10.1. Talent pipetting phosphate-buffered saline into the tube to dilute and adjusting concentration,

2.10.2. Shot of the tube being placed on ice.

3. Adoptive Transfer of CD4⁺ T Cells and Mannan-Induced Inflammation

3.1. For adoptive transfer of CD4⁺ T cells, first gently clean the inner canthus of an anesthetized C57BL/6 mouse with a sterile cotton swab **[1-TXT]**.

3.1.1. Talent using a sterile cotton swab to clean the inner corner of the mouse's eye. **TXT: Perform all steps in a laminar flow hood in an SPF facility**

3.2. Immobilize the mouse by hand **[1]**. Then draw 200 microliters of CD4⁺ T cell suspension, into a 1-milliliter syringe **[2]**.

3.2.1. Talent holding the mouse gently but securely.

3.2.2. Talent drawing 200 microliters of T cell suspension into a syringe.

3.3. Insert the needle at a 10-to-15-degree angle into the inner canthal vein, ensuring accurate placement **[1]**. Inject the suspension slowly and evenly over 10 to 15 seconds **[2]**. After withdrawing the needle, press the inner canthal area gently with a sterile cotton swab for 3 to 5 seconds to prevent bleeding **[3]**.

3.3.1. Shot of the syringe being inserted at a shallow angle into the mouse's inner canthal vein.
AUTHOR'S NOTE: Replace shot 3.3.1 with footage from slate 3.3.1-4.Order and numbering remain same

3.3.2. Talent injecting the cell suspension slowly into the vein.
AUTHOR'S NOTE: Replace shot 3.3.2 with footage from slate 3.3.1-3, Order and numbering remain same

3.3.3. Talent dabbing the injection site gently with a cotton swab after needle removal.

3.4. Now place the mouse in a quiet, dry, and clean cage for monitoring until it fully regains consciousness, with stable breathing and no abnormal behaviour **[1]**. Record the infusion details **[2]** and label the model and control group mice, ensuring four animals per group to avoid confusion **[3]**.
2.4.1. Talent placing the mouse into a fresh cage and closing the lid gently.
NOTE: Shot was accidentally kept as 2.4.1 when it should have been 3.4.1

3.4.1. Talent recording infusion details.

3.4.2. Shot of the mouse groups being labelled model and control.

3.5. Inject CD4⁺ T cells to the model mice, while leaving the control mice untreated **[1-TXT]**.

3.5.1. Talent injecting CD4-positive T cells only into the designated model mice. **TXT: Ensure all animals used are recipient mice**

3.6. On day 4, weigh the mannan powder **[1]** and dissolve it in sterile PBS to a concentration of 100 milligrams per milliliter **[2]**.

3.6.1. Talent weighing mannan powder on a balance.

3.6.2. Talent adding mannan powder to a tube of PBS.

3.7. Hold the mouse securely to expose its abdomen **[1]**. Then disinfect the skin using 75 % alcohol **[2].** Locate the injection site approximately 1 centimetre to the side of the abdominal midline **[3]**.

3.7.1. Talent gripping the mouse to expose the abdominal area.

3.7.2. Talent cleaning the abdomen with alcohol.

3.7.3. Talent indicating the injection point.

3.8. Mix the mannan solution thoroughly **[1]**. Draw 20 to 30 milligrams of the solution into a 1-milliliter syringe **[2]**.

3.8.1. Talent mixing the tube of mannan solution.

3.8.2. Talent drawing the solution into a syringe.

3.9. Insert the needle at a 45-degree angle into the peritoneal cavity and inject slowly to ensure even distribution **[1]**. Withdraw the needle slowly **[2]** and press the injection site gently with a sterile cotton swab for a few seconds **[3]**.

3.9.1. Shot of the needle being injected at a 45-degree angle into the peritoneum.

3.9.2. Shot of the needle being withdrawn slowly.

3.9.3. Talent applying pressure at the injection site with a cotton swab.

3.10. Transfer the mouse into a quiet and clean cage and observe for 5 to 10 minutes to ensure there is no leakage, abdominal distension, or abnormal breathing **[1]**. Record the injection details thoroughly for each mouse **[2]**.

3.10.1. Talent placing the injected mouse into a clean cage and observing its behaviour.

3.10.2. Talent recording the injection details.

Results

4. Representative Results

4.1. The incidence rate in the model group reached 100%, and clinical scores for joint swelling significantly increased over 6 weeks, with a temporary relief observed during the second week **[1]**. Significant thickening and swelling were observed in the forelimb and hindlimb joints of model group mice **[2]**.

4.1.1. LAB MEDIA: Figure 1B

4.1.2. LAB MEDIA: Figure 2. *Video editor: Highlight images in B and C*

4.2. Ankle joint pathology in model group mice revealed pronounced synovial thickening **[1]**, bone discontinuity **[2]**, and marked inflammatory cell aggregation compared to controls **[3]**.

4.2.1. LAB MEDIA: Figure 3 C and Figure 4. *Video Editor: please emphasize areas pointed at by black arrows in 3C and also highlight the red column in Figure 4*

4.2.2. LAB MEDIA: Figure 3B *Video Editor: please emphasize areas pointed at by black arrows in 3B*

4.2.3. LAB MEDIA: Figure 5. *Video editor: Please focus on the areas inside the dotted boxes in 5B*

4.3. Serum analysis revealed that the model group had significantly higher levels of IL-6 *(I-L-six)*, IL-10 *(I-L-ten)*, TNF*(T-N-F)*, IL-17 *(I-L-seventeen)* and IFN-gamma compared to the control group **[1]**. In the spleen, mRNA *(M-R-N-A)* levels of Tbx21 *(T-B-X-Twenty-One)* and IL-17 were significantly elevated in the model group compared to controls, indicating increased Th1 *(T-H-one)* and Th17 *(T-H-Seventeen)* cell activity **[2]**.

4.3.1. LAB MEDIA: Figure 6A. *Video Editor: Please sequentially highlight the red columns of 6A from IL-6 to IFN-gamma*

4.3.2. LAB MEDIA: Figure 6B *Video Editor: Please sequentially highlight the red columns of 6B of TBX21 and IL-17*

Pronunciation Guide:

1. **Rheumatoid Arthritis**
- **Pronunciation link:** Merriam-Webster
- **IPA:** /ˈruːməˌtɔɪd ɑːrˈθraɪtɪs/
- **Phonetic Spelling:** roo-muh-toyd ar-thry-tis

2. CD4
- **Pronunciation link:** Merriam-Webster
- **IPA:** /ˌsiːˌdiːˈfɔːr/
- **Phonetic Spelling:** see-dee-four

3. Mannan
- **Pronunciation link:** Merriam-Webster
- **IPA:** /ˈmænˌæn/
- **Phonetic Spelling:** man-an

4. Synovial
- **Pronunciation link:** Merriam-Webster
- **IPA:** /sɪˈnoʊviəl/
- **Phonetic Spelling:** sih-noh-vee-uhl

5. Peritoneal
- **Pronunciation link:** Merriam-Webster
- **IPA:** /ˌperɪtəˈniːəl/
- **Phonetic Spelling:** peh-rih-toh-nee-uhl

6. Streptavidin
- **Pronunciation link:** Merriam-Webster
- **IPA:** /ˌstrɛptəˈvɪdɪn/
- **Phonetic Spelling:** strep-tuh-vid-in

7. Trypan Blue
- **Pronunciation link:** Merriam-Webster
- **IPA:** /ˈtrɪpæn bluː/
- **Phonetic Spelling:** trip-an blue

8. Tbx21
- **Pronunciation link:** No confirmed link found
- **IPA:** /tiː-biː-ɛks-twɛnti-wʌn/
- **Phonetic Spelling:** tee-bee-ex twenty-one

9. Th17

- **Pronunciation link:** No confirmed link found
- **IPA:** /tiː-ɛɪtʃ ˈsɛvənˈtiːn/
- **Phonetic Spelling:** tee-aitch seventeen