FINAL SCRIPT: APPROVED FOR FILMING

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Title: Murine Model of Epicutaneously-Induced Immunomodulation

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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? **NO**

Current Protocol Length
Number of Steps: 19
Number of Shots: 49

Introduction

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

1.1. **Monika Majewska-Szczepanik:** This protocol outlines a method for skin-induced immunomodulation in a murine model of human allergic contact dermatitis mediated by T helper 1 lymphocytes.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1.*

What are the most recent developments in your field of research?

1.2. **Magdalena Zemelka-Wiącek:** Recent studies show that epicutaneous immunization with protein antigen alone suppresses T cell-mediated immune responses, while co-administration with PAMPs reverses skin-induced suppression. This highlights the potential of skin-based, needle-free strategies for generating regulatory cells to modulate immune responses.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 4.*

What are the current experimental challenges?

1.3. **Magdalena Zemelka-Wiącek:** It is well known that the degree of epidermal damage prior to antigen administration may determine the type of the induced immune response.

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

What significant findings have you established in your field?

1.4. **Monika Majewska-Szczepanik:** Our study has established that epicutaneously-induced immunomodulation is mediated by antigen-non-specific T suppressor cells and antigen-specific T contrasuppressor cells.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 5.*

What research gap are you addressing with your protocol?

1.5. **Magdalena Zemelka-Wiącek:** Despite widespread use of EC immunization, the lack of standardized protocols and variations in application methods create inconsistencies in outcomes. Our protocol addresses these challenges by offering a reproducible and controlled murine model.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.6.1, 2.6.2.*

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

Testimonial Questions:

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Monika Majewska-Szczepanik:** We believe that publishing our method will increase its visibility and encourage other researchers to use it.

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

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE?

1.7. **Magdalena Zemelka-Wiącek:** Presenting our methods and results in JOVE offers an excellent opportunity to enhance collaboration and increase citations for our group. Additionally, our protocol has the potential to reduce training time and costs in other laboratories. We believe that by implementing our protocol, productivity and research outcomes in those labs can be significantly improved.

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

Ethics Title Card

This research has been approved by the 1st and 2nd Local Ethical Committee on Animal Testing in Krakow

2. Induction of Suppressor Cells (Ts) and Testing of Their Role in Th1-Mediated CHS Reaction

Demonstrators: Monika Majewska-Szczepanik and Katarzyna Marcińska

2.1. To begin, shave the back skin of the anesthetized mouse with a razor on day 0 *(zero)*. **[1-TXT]**.

2.1.1. WIDE: Talent shaving the back skin with the razor. **TXT: Anesthesia: 30% isoflurane in propylene glycol; After shaving, wait at least 6 h before applying the antigen** *Videographer: Film this shot as a combination of the talent shaving the mouse and a close-up shot of the shaving.*
Videographer's Note: shot 2.1.1 with additional 2.1.1.A shot describing this same procedure-. Please use both of them

2.2. On day 1, prepare a 1 milligram per milliliter solution of antigen 2,4,6-trinitrophenyl-conjugated mouse immunoglobulin in sterile DPBS *(D-P-B-S)* **[1]**. Protect the antigen from light by covering the vial with aluminum foil **[2]**. Mix the solutions just before use in a 2-milliliter vial **[3]**.

2.2.1. Shot of the prepared antigen solution placed on the lab bench or a biosafety hood and the talent standing beside it. **TXT: Prepare 1200 µL antigen for 10 mice**
Videographer's Note: shot 2.2.1 with additional 2.2.1.A and 2.2.1.B shot describing this same procedure- please use all of them

2.2.2. Talent covering the vial with aluminum foil.

2.2.3. Talent mixing the solution in a 2-milliliter vial.

2.3. Apply the antigen solution to the shaved skin by placing a 1 square centimeter gauze patch soaked in 100 microliters of antigen **[1-TXT]**. Cover this gauze with a 2 square centimeter thin plastic patch and secure it with fabric adhesive tape **[2]**. Once the mouse recovers from anesthesia, return it to its cage **[3]**.

2.3.1. Talent placing an antigen-soaked gauze patch onto the shaved area of the mouse skin. **TXT: Apply only DPBS to the contact hypersensitivity control group**

2.3.2. Talent covering the gauze with a plastic patch and securing it with fabric adhesive tape.

2.3.3. Talent placing the mouse into the cage and the mouse moving freely in the cage.

2.4. On day 5, after anesthetizing the mouse, use safety scissors with rounded corners to carefully cut the patch **[1]**. Gently remove the adhesive tape **[2]**. After the mouse regains consciousness and is returned to its cage, allow it to move freely and clean its skin naturally for at least 4 hours **[3-TXT]**.

2.4.1. Talent cutting the patch carefully with rounded scissors.

2.4.2. Talent removing the tape from the mouse's skin.

2.4.3. Wide shot of the mouse moving freely in the cage. **TXT: As the patch must remain on the skin for 7 d, reapply the antigen solution on day 5 and remove on day 8**

2.5. On day 8, to test EC *(E-C)*-induced T suppressor cells in Th1 *(T-H-one)*-mediated contact hypersensitivity reaction, apply 150 microliters of the freshly prepared TNCB *(T-N-C-B)* hapten solution to the shaved abdominal skin of the anesthetized mouse to induce T effector cells **[1-TXT]**.

2.5.1. Talent applying the hapten solution onto the shaved abdomen area of the mouse skin using a pipette. **TXT: In donors of Teffector cells do this step on Day 0**

2.6. Four days after inducing Teffector *(T-effector)* cells, evaluate the contact hypersensitivity response by measuring the ear baseline and applying TNCB *(T-N-C-B)* hapten to both sides of the ears **[1]**. Measure the ear thickness by using a micrometer 24 hours later **[2]**.

2.6.1. Talent measuring the ear baseline and applying TNCB on both sides of mouse ears.
Videographer's Note: do not use shot no.2.6.1 use 2.6.1.A and 2.6.1.B instead

2.6.2. Talent measuring ear thickness using a micrometer.

3. Isolation of Teff orTs Cells from Donor Mice

3.1. For the isolation of 7-day-Tsuppressor *(T-suppressor)*, or 4-day-T effector cells from the donor mice, disinfect the entire abdominal area of the anesthetized mouse with 70 percent ethanol **[1-TXT]**. Make a 1-centimeter incision to expose the peritoneal cavity **[2]**. Using sterile forceps, isolate the auxiliary and inguinal lymph nodes and spleen into tubes filled with sterile DPBS supplemented with 1 percent fetal bovine serum or FBS *(F-B-S)* **[3]**. Pool the lymph nodes from all donors in one vial and spleens in another, and keep the vials on ice **[4]**.

3.1.1. Talent disinfecting the mouse abdomen with ethanol. **TXT: Anesthesia: Ketamine (90–120 mg/ kg) & xylazine (5–10 mg/ kg), I.P.**
Videographer's Note: Also recorded as 4.6.1. Please use one of them

3.1.2. Talent making an abdominal incision with sterile tools.
Videographer's Note: Also recorded as 4.6.2. Use one of them (cut early)

3.1.3. Talent transferring lymph nodes and spleen into labeled tubes containing supplemented buffer.
Videographer's Note: Also recorded as 4.6.3. Use one of them with additional 3.1.3A shot

3.1.4. A shot of the two vials (one containing lymph nodes and the other containing the spleens) placed on ice.
Videographer's Note: Also recorded as 4.6.4. Use one of them

3.2. Mash lymph nodes and spleens between the frosted ends of two microscope slides to create a cell suspension **[1]**. Strain the cell suspension through a cell strainer with a 70-micrometer pore size **[2]**.

3.2.1. Talent crushing tissue between slides.
Videographer's Note:shot 3.2.2 with additional 3.2.2.A shot describing this same procedure-please use all of them.

3.2.2. Talent pouring suspension through the cell strainer.

3.3. Rinse the cells with 30 milliliters of DPBS supplemented with 1 percent FBS **[1]** and centrifuge at 300 *g* for 10 minutes at 4 degrees Celsius **[2]**. Discard the supernatant **[3]** and resuspend the pellet in 1 to 5 milliliters of DPBS **[4]**.

3.3.1. Talent washing the cell pellet with buffer.

3.3.2. Talent placing tubes in the centrifuge.

3.3.3. Talent discarding the supernatant.

3.3.4. Talent resuspending the final pellet in DPBS.

3.4. To count the cell viability, mix 10 microliters of cell suspension with 90 to 990 microliters of Trypan Blue, depending on the cell count **[1]**, and count the cells using a hemocytometer **[2]**.

3.4.1. Talent mixing 10 microliters of cell suspension with 90 to 990 microliters of Trypan Blue.

3.4.2. Talent counting cells under a microscope with hemocytometer.

3.5. Next, prepare a 1:1 *(one to one)* mixture of auxiliary lymph node and spleen cells **[1]**to obtain 1 to 5 × 10^7 Tsuppressor *(T-Suppressor)* cells per recipient in 1 milliliter of DPBS with 1 percent FBS **[2]**. For T-effector cells, prepare 7 × 10^7 cells per recipient in 1 milliliter of the same buffer **[3]**.

Videographer's Note:shots 3.5.1 and 3.5.2 and 3.5.3 recorded together in one take

3.5.1. Talent mixing equal volumes of lymph node and spleen cells.

3.5.2. Talent adding Tsuppressor cells in DPBS with 1 percent FBS.

3.5.3. Talent adding T-effector cells in DPBS with 1 percent FBS.

4. Adoptive Transfer IN and OUT: Testing of Ts in Th1-Mediated CHS

4.1. Rinse the isolated cells with 30 milliliters of DPBS **[1]**. Then, centrifuge them at 300 *g* for 10 minutes at 4 degrees Celsius **[2]** and resuspend the pellet in 200 microliters of DPBS per recipient **[3]**.

4.1.1. Talent washing the cells with DPBS.

4.1.2. Talent centrifuging the cells.

4.1.3. Talent resuspending the cells in DPBS.

4.2. For the adoptive transfer IN *(in)*, administer an intravenous injection of the prepared mixture containing 1 to 5 × 10^7 Tsuppressor cells in 200 microliters to the anesthetized recipient before inducing Th1-mediated contact hypersensitivity reaction **[1-TXT]**. On the same day, sensitize anesthetized mice by applying 150 microliters of hapten on the previously shaved spot **[2]**. Four days later, evaluate the contact hypersensitivity response by measuring the ear baseline **[3]** and applying hapten to both sides of the ears **[4]**. Measure the ear thickness 24 hours later **[5]**.

4.2.1. Talent injecting prepared Ts cells into the anesthetized mouse. **TXT: Anesthesia: Ketamine (90–120 mg/ kg) & xylazine (5–10 mg/ kg), I.P. : Do this step on Day 0**

4.2.2. Talent applying the hapten solution onto the shaved area of the mouse skin using a pipette.

4.2.3. Talent measuring the ear baseline.

4.2.4. Talent applying hapten on the ears.

4.2.5. Talent measuring the ear thickness.

4.3. For the adoptive transfer OUT *(out)*, after inducing and isolating Tsuppressor and Teffector cells from donors, divide the Teffector cells into two separate vials labeled as

"Ts *(T-S)* test group" and "CHS *(C-H-S)* control" **[1-TXT]**.

4.3.1. A shot of Teffector cells into two separate vials labeled as "Ts test group" and "CHS control". **TXT: Use 7 × 10^7 cells in 1 ml per recipient**

4.4. Add Tsuppressor cells to Teffector cells for the "Ts test group" **[1-TXT]** and mix gently **[2]**. Then, add DPBS supplemented with 1% FBS only to the Teffector cells in the "CHS control" vial **[3]** and mix it **[4]**.

4.4.1. Talent adding T-suppressor cells to T-effector cells in the "Ts test group" vial. **TXT: Use 1 to 5 × 10^7 cells in 1 ml per recipient**

4.4.2. Talent mixing the contents in "Ts test group" vial.

4.4.3. Talent adding DPBS with 1% FBS to "CHS control" vial containing Teffector cells.
Videographer's Note:shots 4.4.3 and 4.4.2 recorded together in one take

4.4.4. Talent mixing the contents in "CHS control" vial.
Videographer's Note:shots 4.4.4 and 4.4.2 recorded together in one take

4.5. Place the vials in a 37 degrees Celsius water bath for 30 minutes **[1]**, mixing the contents gently every 5 minutes **[2]**.

4.5.1. Talent placing vials in water bath and setting the timer.

4.5.2. Talent mixing the contents of the vials.

4.6. Rinse each vial with 30 milliliters of DPBS **[1]**. Then, centrifuge at 300 *g* for 10 minutes at 4 degrees Celsius **[2]**. Decant the supernatant **[3]** and resuspend the pellet in 200 microliters of DPBS per recipient **[4]**.
Videographer's Note: Pleas use shots named X instead of original shot names for 4.6.1-4.6.4. Ex: Use 4.6.1X instead of 4.6.1

4.6.1. Talent rinsing the vial with DPBS.

4.6.2. Talent performing centrifugation.

4.6.3. Talent decanting the supernatant.

4.6.4. Talent resuspending pellet in DPBS.

4.7. Now, pass each cell suspension through a 70-micrometer pore cell strainer **[1]**.
Videographer's Note: Use 4.7.1X instead of 4.7.1

4.7.1. Talent filtering the cell suspension.

4.8. Administer an intravenous injection of the prepared cells into recipient mice **[1-TXT]**.

4.8.1. Talent injecting mice with the prepared cells. **TXT: On the same day, induce the elicitation phase of contact hypersensitivity and include a negative control**

Results

5. Results

5.1. Epicutaneous exposure to antigen with or without pathogen-associated molecular pattern influences the induction and modulation of contact hypersensitivity reaction **[1]**.

5.1.1. LAB MEDIA: Figure 4.

5.2. Epicutaneous immunization with TNP-Ig *(T-N-P I-G)* antigen significantly suppressed contact hypersensitivity as shown by reduced ear swelling compared to the contact hypersensitivity control group **[1]**. Co-application with lipopolysaccharide reversed this suppression, restoring swelling levels similar to the control **[2]**.

5.2.1. LAB MEDIA: Figure 4A. *Video Editor: Highlight the top grey bar.*

5.2.2. LAB MEDIA: Figure 4B. *Video Editor: Highlight the second grey bar from top.*

5.3. Intravenous transfer of lymph node and spleen cells from mice tolerized with TNP-Ig significantly reduced TNCB *(T-N-C-B)* induced ear swelling in recipient mice compared to the contact hypersensitivity control, confirming the suppressive role of T suppressor cells **[1]**.

5.3.1. LAB MEDIA: Figure 5. *Video Editor: Highlight the top grey bar.*

5.4. Transfer of T effector cells incubated with T suppressor cells significantly suppressed the contact hypersensitivity response in recipients, while transfer of T effector cells alone triggered the contact hypersensitivity response **[1]**.

5.4.1. LAB MEDIA: Figure 6. *Video Editor: Highlight the two grey bars at the top.*

Pronunciation Guide:

 murine
Pronunciation link: https://www.merriam-webster.com/dictionary/murine
IPA: /ˈmjuːˌraɪn/
Phonetic Spelling: MYOO-ryne

 epicutaneously / **epicutaneous**
Pronunciation link: https://www.collinsdictionary.com/us/dictionary/english/epicutaneous
IPA: /ˌɛpɪkjuːˈteɪniəs/ (for the adjective)
Phonetic Spelling: ep-i-kyoo-TA-nee-uhs
(for the adverb "epicutaneously," add "-lee" at end: ep-i-kyoo-TA-nee-uhs-lee)

 immunomodulation
Pronunciation link: https://www.howtopronounce.com/immunomodulation
IPA: /ɪˌmjuːnoʊˌmɒdʒəˈleɪʃən/
Phonetic Spelling: im-yoo-noh-modj-uh-LAY-shun

 antigen
Pronunciation link: https://www.merriam-webster.com/dictionary/antigen
IPA: /ˈæntəˌdʒən/
Phonetic Spelling: AN-ti-jən

 PAMPs (Pathogen-Associated Molecular Patterns)

- **pathogen**
  Pronunciation link: https://www.merriam-webster.com/dictionary/pathogen
  IPA: /ˈpæθədʒən/
  Phonetic Spelling: PATH-uh-jən
- **associated**
  Pronunciation link: https://www.merriam-webster.com/dictionary/associated
  IPA: /əˈsoʊʃiˌeɪtɪd/
  Phonetic Spelling: uh-SOH-shee-ay-tid
- **molecular**
  Pronunciation link: https://www.merriam-webster.com/dictionary/molecular
  IPA: /məˈlɛkjələr/
  Phonetic Spelling: muh-LEK-yuh-lər
- **pattern(s)**
  Pronunciation link: https://www.merriam-webster.com/dictionary/pattern
  IPA: /ˈpætərn/
  Phonetic Spelling: PAT-ərn

 T suppressor / **T contrasuppressor**

- **suppressor**
  Pronunciation link: https://www.howtopronounce.com/suppressor
  IPA: /səˈprɛsər/
  Phonetic Spelling: suh-PRESS-ər
- **contra-suppressor**
  (Just attach "contra-" as /ˈkɑntrə/ + suppressor) → IPA: /ˈkɑntrə səˈprɛsər/
  Phonetic Spelling: KON-truh suh-PRESS-ər

- **hypersensitivity**
Pronunciation link: https://www.merriam-webster.com/dictionary/hypersensitivity
IPA: /ˌhaɪpərsɛnˈsɪvɪti/
Phonetic Spelling: hy-per-sen-SIH-vuh-tee
- **adoptive**
Pronunciation link: https://www.merriam-webster.com/dictionary/adoptive
IPA: /əˈdɑptɪv/
Phonetic Spelling: uh-DOP-tiv
- **intravenous**
Pronunciation link: https://www.merriam-webster.com/dictionary/intravenous
IPA: /ˌɪntrəˈviːnəs/
Phonetic Spelling: in-truh-VEE-nuhs
- **isoflurane**
Pronunciation link: https://www.howtopronounce.com/isoflurane
IPA: /ˌaɪsoʊˈflʌreɪn/
Phonetic Spelling: eye-soh-FLOOR-ayn
- **xylazine**
Pronunciation link: https://www.howtopronounce.com/xylazine
IPA: /zaɪˈleɪziːn/
Phonetic Spelling: zy-LAY-zeen
- **micrometer**
Pronunciation link: https://www.merriam-webster.com/dictionary/micrometer
IPA: /ˈmaɪkrəˌmiːtər/
Phonetic Spelling: MY-kruh-mee-ter