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Title: Establishment of a Mouse Sepsis-Induced Immunosuppression Model by Intranasal Instillation of Bacteria

Authors and Affiliations:

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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**
If **Yes**, how far apart are the locations? Within 500 meters.
- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 10

Number of Shots: 22

Interviews

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

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

Please note that the answers are edited in accordance with journal guidelines.

INTRODUCTION:

~~**REQUIRED:** Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Kaiwei Cao:** The aim of this study is to establish a sepsis immunosuppression model through natural infection pathways.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~**Describe the research contexts or systems in which this protocol can be applied.**~~

- 1.2. **Kaiwei Cao:** This experimental method is applicable for the study of molecular mechanisms and treatment strategies related to sepsis.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~**What does this protocol allow researchers to study or measure?**~~

- 1.3. **Qinyuan Li:** This protocol allows evaluation of organ damage and molecular mechanisms of immune suppression in sepsis under natural infection pathways.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~**Describe the most important consideration or challenge when performing this protocol.**~~

- 1.4. **Xifeng Feng:** The most important consideration when performing this protocol is to ensure complete inhalation of bacterial solution.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Qinyuan Li**: Future studies can establish an array of immunosuppressive models using different bacterial species.
 - 1.5.1. 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 Laboratory Animal Welfare and Ethics Committee of the Third Military Medical University

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Intranasal Bacterial Instillation in Mice

Demonstrators: Kaiwei Cao, Xifeng Feng, Qinyuan Li, Yuanyuan He

- 2.1. To begin, obtain the *Klebsiella pneumoniae* ATCC 43816 (4-3-8-1-6) [1] and dilute the culture in 0.9 percent sodium chloride solution to prepare the bacterial suspensions at the required concentrations [2-TXT].
 - 2.1.1. WIDE: Talent placing the bacterial culture in the hood. ***Videographer:** Please obtain CU shot as well for this shot.*
 - 2.1.2. Talent adding the culture to tubes containing 0.9%NaCl. **TXT: Bacterial concentrations: 1×10^8 CFU/ml; 1×10^6 CFU/mL; and 1×10^4 CFU/mL**
- 2.2. Place an 8 to 10-week-old male C57BL-6 mouse on a clean work surface [1] and inject 2 percent sodium pentobarbital at a dose of 2 microliters per gram body weight intraperitoneally [2].
 - 2.2.1. Talent positioning the mouse on the table for injection.
 - 2.2.2. Talent administering the intraperitoneal injection to anesthetize the mouse.
- 2.3. Aspirate 50 microliters of the bacterial suspension using a micropipette when the mouse exhibits reduced consciousness but retains the swallowing reflex [1-TXT].
 - 2.3.1. Talent aspirating 50 microliters of the bacterial suspension or control solution using a micropipette. **TXT: Control: 0.9% NaCl**
- 2.4. Gently hold the mouse's neck with the thumb placed on the front of the neck [1] and gently grasp the mouse's body with the remaining fingers [2]. Tilt the mouse's head backward to facilitate intranasal instillation [3].
 - 2.4.1. Talent gently holding the mouse's neck with the thumb placed on the front of the neck.
 - 2.4.2. Talent gently grasping the mouse's body with the remaining fingers.
 - 2.4.3. Talent tilting the mouse's head backward.

- 2.5. Next, slowly instill the bacterial suspension into the nostrils in 5 to 10 microliter drops [1], administering one drop into one nostril at a time [2].
 - 2.5.1. Talent slowly instilling the bacterial suspension into the mouse's nostril.
 - 2.5.2. Close-up of only 1 drop falling into a nostril.
- 2.6. After confirming that the mouse has inhaled the suspension [1], quickly lift the mouse upward [2]. Then slowly lower the mouse to promote inhalation of suspension into the bronchi [3].
 - 2.6.1. Talent observing as the mouse inhales the suspension.
 - 2.6.2. Talent quickly lifting the mouse upward.
 - 2.6.3. Talent slowly lowering the mouse.
- 2.7. Following instillation, position the mouse supine on a board tilted at 60 degrees [1]. If necessary, provide supplemental heat [2]. After the animal fully recovers from anesthesia [3], return it to its cage with optimal temperature conditions, sufficient water, and food [4].
 - 2.7.1. Talent fixing the mouse in a supine position on a mouse board tilted at 60 degrees.
 - 2.7.2. Talent turning on a heater to help the mouse recover.
 - 2.7.3. Shot of the mouse showing movements after recovery.
 - 2.7.4. Talent returning the recovered mouse to its cage.

Authors: The following steps were **not** highlighted by you and we understand that you did not want to film these. But we have added them as representative shots to bridge the results to the protocol

- 2.8. Record the body weight, hair condition, activity level, and survival daily [1]. Collect blood samples at 12 and 24 hours after instillation to evaluate the inflammatory and organ-damage markers [2-TXT].
 - 2.8.1. Talent recording the animal's weight by placing it on a weighing scale and noting the weight in a book.
 - 2.8.2. Talent showing a vial with blood. **TXT: Repeat blood collection 7 days after instillation** **Authors:** You need **not** euthanize the animal or show collecting blood from the animal for this. Please show a tube with any blood sample you may have or a red solution that mimics blood. *Videographer: Please obtain WIDE and CU shot as well for this shot*

2.9. Assess immune cell changes by flow cytometry [1].

2.9.1. Talent loading a sample into the flow cytometer.

2.10. On day 7 after model establishment, subject the mouse to intranasal instillation of 50 microliters of *Pseudomonas aeruginosa* or PA following the same procedure as demonstrated earlier [1].

2.10.1. *Reuse 2.5.1 and 2.5.2.* **TXT: PA concentration: 6.8×10^{10} CFU/mL**

Results

3. Results

3.1. At 12 hours after intranasal instillation, the serum interleukin-6 level was significantly increased in the 1×10^8 KP group [1-TXT].

3.1.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the bar labeled 1×10^8 KP.* **TXT:**
KP: *Klebsiella pneumoniae*

3.2. At 24 hours after intranasal instillation, the serum alanine aminotransferase, aspartate aminotransferase, creatine kinase, and blood urea nitrogen levels were significantly higher in the 1×10^8 KP group [1] than in the control group [2].

3.2.1. LAB MEDIA: Figures 2B–E. *Video editor: Highlight the bars labeled 1×10^8 KP.*

3.2.2. LAB MEDIA: Figures 2B–E. *Video editor: Highlight the bars labeled control.*

3.3. On day 3 after intranasal instillation, body weights significantly decreased in mice from the 1×10^6 [1] and the 1×10^8 KP group [2].

3.3.1. LAB MEDIA: Figure 2F. *Video editor: Highlight the day 3 point for the line labeled 1×10^6 KP.*

3.3.2. LAB MEDIA: Figure 2F. *Video editor: Highlight the day 3 point for the line labeled 1×10^8 KP.*

3.4. On day 7 after intranasal instillation, the 1×10^8 KP group showed a significantly decreased lymphocyte percentage [1] and the proportion of major histocompatibility complex class II (2) expression in monocytes [2] compared with the control group [3].

3.4.1. LAB MEDIA: Figure 3AB. *Video editor: Show the bar graphs. Highlight the bar labeled 1×10^8 KP in A graph.*

3.4.2. LAB MEDIA: Figure 3AB. *Video editor: Show the bar graphs. Highlight the bar labeled 1×10^8 KP in B graph.*

3.4.3. LAB MEDIA: Figure 3AB. *Video editor: Show the bar graphs. Highlight the control bars.*

3.5. The mean fluorescence intensities of interleukin-10 [1] and transforming growth factor

beta 1 in CD4-positive T cells were significantly increased in the 1×10^8 KP group [2].

3.5.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the bar labeled 1×10^8 KP.*

3.5.2. LAB MEDIA: Figure 4C. *Video editor: Highlight the bar labeled 1×10^8 KP.*

3.6. At 24 hours after secondary infection with *Pseudomonas aeruginosa*, the bacterial load in bronchoalveolar lavage fluid was significantly increased in mice from the 1×10^8 KP group [1].

3.6.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the bar labeled 1×10^8 KP.*

3.7. Within 7 days after intranasal instillation, mortality rates were significantly increased in mice from the 1×10^6 KP group [1] and the 1×10^8 KP group [2].

3.7.1. LAB MEDIA: Figure 4D. *Video editor: Highlight the survival line labeled 1×10^6 KP.*

3.7.2. LAB MEDIA: Figure 4D. *Video editor: Highlight the survival line labeled 1×10^8 KP.*

1. Klebsiella

Pronunciation link: <https://www.merriam-webster.com/dictionary/Klebsiella>

IPA: /ˌkleb-si-'el-ə/

Phonetic Spelling: kleb·see·el·uh

2. pneumoniae

Pronunciation link:

<https://www.howtopronounce.com/pneumoniae>

IPA: /nu:'moʊni.i:/

Phonetic Spelling: noo·moh·nee·ee

3. ATCC

Pronunciation link: No confirmed link found

IPA: /ˌeɪ ti: si: 'si:/

Phonetic Spelling: ay·tee·see·see

4. sodium chloride

Pronunciation link: <https://www.merriam-webster.com/dictionary/sodium>

IPA: /'soʊdiəm/ + /'klɔːraɪd/

Phonetic Spelling: soh·dee·uhm klor·ide

5. bacterial

Pronunciation link: <https://www.merriam-webster.com/dictionary/bacterial>

IPA: /bæk'tɪriəl/

Phonetic Spelling: bak·teer·ee·uhl

6. suspension

Pronunciation link: <https://www.merriam-webster.com/dictionary/suspension>

IPA: /sə'spenʃən/

Phonetic Spelling: suh·spen·shuhn

7. concentration

Pronunciation link: <https://www.merriam-webster.com/dictionary/concentration>

IPA: /ˌkɑːn.sən'treɪ.ʃən/

Phonetic Spelling: kon·suhn·tray·shuhn

8. C57BL-6

Pronunciation link: No confirmed link found

IPA: /ˌsiː ˌfɪf.ti 'sev.ən biː 'el sɪks/

Phonetic Spelling: see·fif·tee·sev·uhn·bee·el·siks

9. pentobarbital

Pronunciation link: <https://www.merriam-webster.com/dictionary/pentobarbital>

IPA: /ˌpen.toʊ'bɑːr.bɪ.tɑːl/

Phonetic Spelling: pen·toh·bar·bih·taal

10. microliters

Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>

IPA: /'maɪkroʊ.li:tər/

Phonetic Spelling: my·kroh·lee·ter

11. intraperitoneally

Pronunciation link:

<https://www.howtopronounce.com/intraperitoneally>

IPA: /ˌɪn.trəˈper.ɪ.təˈniː.ə.li/

Phonetic Spelling: in·truh·pair·ih·tuh·nee·uh·lee

12. aspirate

Pronunciation link: <https://www.merriam-webster.com/dictionary/aspirate>

IPA: /ˈæs.pəˈreɪt/

Phonetic Spelling: ass·puh·rayt

13. micropipette

Pronunciation link:

<https://www.howtopronounce.com/micropipette>

IPA: /ˌmaɪ.kroʊ.paiˈpet/

Phonetic Spelling: my·kroh·pie·pet

14. consciousness

Pronunciation link: <https://www.merriam-webster.com/dictionary/consciousness>

IPA: /ˈkɑːnʃəsnəs/

Phonetic Spelling: kon·shuhs·nuhs

15. swallowing

Pronunciation link: <https://www.merriam-webster.com/dictionary/swallowing>

IPA: /ˈswɑːləʊɪŋ/

Phonetic Spelling: swah·loh·ing

16. intranasal

Pronunciation link: <https://www.merriam-webster.com/dictionary/intranasal>

IPA: /ˌɪn.trəˈnei.zəl/

Phonetic Spelling: in·truh·nay·zuhl

17. instillation

Pronunciation link: <https://www.merriam-webster.com/dictionary/instillation>

IPA: /ˌɪn.stəˈleɪ.ʃən/

Phonetic Spelling: in·stuh·lay·shuhn

18. nostrils

Pronunciation link: <https://www.merriam-webster.com/dictionary/nostril>

IPA: /ˈnɑːstrəl/

Phonetic Spelling: nahs·truhl

19. bronchi

Pronunciation link: <https://www.merriam-webster.com/dictionary/bronchi>

IPA: /ˈbrɑːŋkaɪ/

Phonetic Spelling: brong·kye

20. supine

Pronunciation link: <https://www.merriam-webster.com/dictionary/supine>

IPA: /ˈsuːpaɪn/

Phonetic Spelling: soo·pine

21. anesthesia

Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthesia>

IPA: /ˌænəsˈθiːzə/, /ˌænəsˈθiːziə/

Phonetic Spelling: an·uhs·thee·zhuh; an·uhs·thee·zee·uh

22. inflammatory

Pronunciation link: <https://www.merriam-webster.com/dictionary/inflammatory>

IPA: /ɪnˈflæməˌtɔːri/

Phonetic Spelling: in·flam·uh·tor·ee

23. organ-damage

Pronunciation link: <https://www.merriam-webster.com/dictionary/organ>

IPA: /ˈɔːrgən/ + /ˈdæmɪdʒ/

Phonetic Spelling: or·guhn dam·ij

24. flow cytometry

Pronunciation link: <https://www.merriam-webster.com/dictionary/cytometry>

IPA: /saɪˈtɑːmətri/

Phonetic Spelling: sigh·tom·uh·tree

25. cytometer

Pronunciation link:

<https://www.howtopronounce.com/cytometer>

IPA: /saɪˈtɑːmɪtər/

Phonetic Spelling: sigh·tom·ih·ter

26. Pseudomonas

Pronunciation link: <https://www.merriam-webster.com/dictionary/Pseudomonas>

IPA: /ˌsuːdəˈmoʊnəs/

Phonetic Spelling: soo·duh·moh·nuhs

27. aeruginosa

Pronunciation link:

<https://www.howtopronounce.com/aeruginosa>

IPA: /əˌruːdʒɪˈnoʊsə/

Phonetic Spelling: uh·roo·jih·noh·suh

28. serum

Pronunciation link: <https://www.merriam-webster.com/dictionary/serum>

IPA: /ˈsɪrəm/

Phonetic Spelling: seer·uhm

29. interleukin-6

Pronunciation link: <https://www.merriam-webster.com/dictionary/interleukin>

IPA: /ˌɪn.təˈluː.kɪn/

Phonetic Spelling: in·ter·loo·kin

30. alanine

Pronunciation link: <https://www.merriam-webster.com/dictionary/alanine>

IPA: /'æləˌniːn/

Phonetic Spelling: al·uh·neen

31. aminotransferase

Pronunciation link: <https://www.howtopronounce.com/aminotransferase>

IPA: /əˌmiː.nʊˈtræns.fəˌreɪs/

Phonetic Spelling: uh·mee·noh·trans·fuh·rays

32. aspartate

Pronunciation link: <https://www.merriam-webster.com/dictionary/aspartate>

IPA: /əˈspɑːrteɪt/

Phonetic Spelling: uh·spar·tayt

33. creatine kinase

Pronunciation link: <https://www.merriam-webster.com/dictionary/creatine>

IPA: /'kriːəˌtiːn/ + /'kaɪˌneɪs/

Phonetic Spelling: kree·uh·teen kye·nays

34. urea

Pronunciation link: <https://www.merriam-webster.com/dictionary/urea>

IPA: /jʊˈriːə/

Phonetic Spelling: yoor·ee·uh

35. lymphocyte

Pronunciation link: <https://www.merriam-webster.com/dictionary/lymphocyte>

IPA: /'lɪmfəˌsaɪt/

Phonetic Spelling: lim·fuh·site

36. histocompatibility

Pronunciation link: <https://www.merriam-webster.com/dictionary/histocompatibility>

IPA: /ˌhɪstəʊkəmˌpætəˈbɪləti/
Phonetic Spelling: hiss-toh-kuhm-pat-uh-bil-uh-tee

37. monocytes

Pronunciation link: <https://www.merriam-webster.com/dictionary/monocyte>
IPA: /ˈmɑːnəˌsaɪt/
Phonetic Spelling: mon-uh-site

38. fluorescence

Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
IPA: /flʊˈresəns/, /flɔːˈresəns/
Phonetic Spelling: floor-ess-uhns

39. intensities

Pronunciation link: <https://www.merriam-webster.com/dictionary/intensity>
IPA: /ɪnˈtensəti/
Phonetic Spelling: in-ten-suh-tee

40. interleukin-10

Pronunciation link: <https://www.merriam-webster.com/dictionary/interleukin>
IPA: /ˌɪn.t̬əˈluː.kɪn/
Phonetic Spelling: in-ter-loo-kin

41. transforming growth factor beta 1

Pronunciation link: <https://www.merriam-webster.com/dictionary/transforming>
IPA: /trænsˈfɔːrmɪŋ/; /groʊθ/; /ˈfæktər/; /ˈbɛtə/
Phonetic Spelling: trans-for-ming grohth fak-ter bay-tuh

42. bronchoalveolar

Pronunciation link: <https://www.howtopronounce.com/bronchoalveolar>
IPA: /ˌbrɑːŋ.koʊ.ælˈviː.ə.lə/
Phonetic Spelling: brong-koh-al-vee-uh-ler

43. lavage

Pronunciation link: <https://www.merriam-webster.com/dictionary/lavage>

IPA: /ləˈvɑːʒ/

Phonetic Spelling: luh·vahzh

44. mortality

Pronunciation link: <https://www.merriam-webster.com/dictionary/mortality>

IPA: /mɔːrˈtæləti/

Phonetic Spelling: mor·tal·uh·tee