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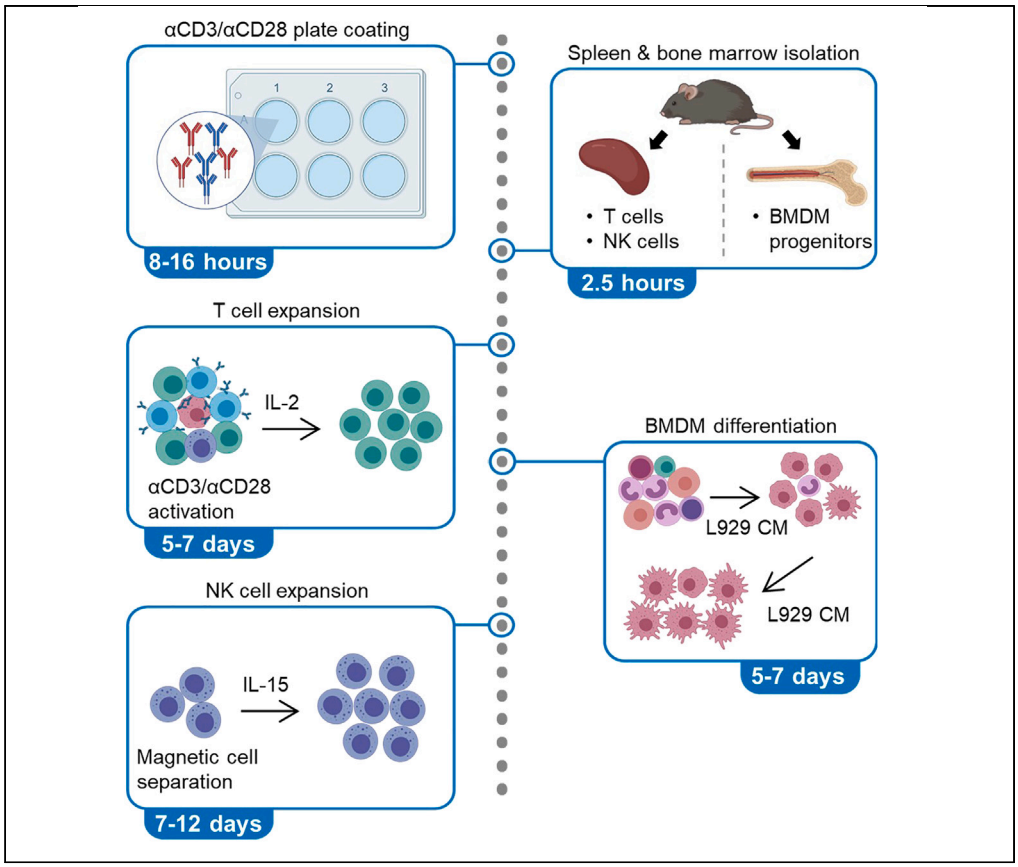
Protocol for the expansion of mouse immune effector cells for *in vitro* and *in vivo* studies



Reproducible and efficient expansion of different immune effector cells is required for pre-clinical studies investigating adoptive cell therapies against cancer. Here, we provide a protocol for the rapid expansion of mouse T cells, natural killer (NK) cells, and bone-marrow-derived macrophages (BMDMs). We describe steps for αCD3/αCD8 plate coating, isolating splenocytes, and expanding T cells and NK cells. Further, we detail procedures for bone marrow isolation and BMDM differentiation.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

Coat plates for T cell activation with αCD3/αCD8 antibodies overnight

Isolate splenocytes and bone marrow cells from mice

Expand mouse T cells, NK cells, and bone-marrow-derived macrophages

Use expanded immune effector cells for *in vitro* or *in vivo* studies

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Protocol for the expansion of mouse immune effector cells for *in vitro* and *in vivo* studies

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SUMMARY

Reproducible and efficient expansion of different immune effector cells is required for pre-clinical studies investigating adoptive cell therapies against cancer. Here, we provide a protocol for the rapid expansion of mouse T cells, natural killer (NK) cells, and bone-marrow-derived macrophages (BMDMs). We describe steps for αCD3/αCD8 plate coating, isolating splenocytes, and expanding T cells and NK cells. Further, we detail procedures for bone marrow isolation and BMDM differentiation.

BEFORE YOU BEGIN

The current protocol enables a rapid and efficient expansion of murine T cells, NK cells and macrophages. These expansion procedures necessitate access to C57BL/6 mice for the extraction of spleens and bone marrow.

For T cell expansion αCD3 and αCD28 antibodies (Bio X Cell, #BE0001-1 and #BE0015-1) as well as mouse IL-2 are required.[1] NK cell isolation and expansion requires the EasySep Mouse NK Cell Isolation Kit (STEMCELL Technologies, #19855)[2] and mouse interleukin (IL)-15.[3] BMDM are differentiated from bone marrow cells using L929 conditioned medium as supplement.[4] L929 conditioned medium from the L929 fibroblast cell line needs to be prepared in advance. Alternatively, mouse macrophage colony-stimulating factor (M-CSF) can be used.

Before starting organ extractions prepare cytokine stocks of mouse IL-2, IL-15, if required M-CSF and freeze them at −80°C. Primary cell cultures described in this protocol require a strictly controlled environment for cell growth. A temperature of 37°C and a CO_2 level of 5% should be maintained in the incubator during cell expansion. To prevent contamination and to ensure comparable functional states of immune effector cells it is required to ensure aseptic working conditions throughout the protocol.

All needed instruments, material and reagents should be purchased from well-established sources to ensure the integrity and reliability of the protocol.

Institutional permissions

All experiments were done in accordance with the guidelines of the Swiss federal law on animal protection and were approved by the cantonal veterinary office. Anyone who is interested in conducting described experiments needs to acquire permission from the relevant institutions before.

Preparation of L929 conditioned medium

Timing: 10 days

1. Thaw L929 cells and seed 5 × 10^6 cells in DMEM/F-12 complete medium in a T-150 flask for adherent cells and grow until confluent.
2. Remove the supernatant, wash 1× with DPBS and detach with TrypLE or Trypsin.
3. Use new T-150 flasks for adherent cells and re-seed 5 × 10^6 cells per flask with 35 mL of DMEM/F-12 complete medium.
4. After 7 days, transfer the medium from the flasks to 50 mL centrifuge tubes and centrifuge at 400 g for 5 min.
5. Collect the supernatant in 15 mL or 50 mL centrifuge tubes and store at −20°C or −80°C.
6. Repeat steps 3–5 if more L929-conditioned medium is needed.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
*InVivo*MAb anti-mouse CD3ε (clone 145-2C11)	Bio X Cell	Cat#BE0001-1
*InVivo*MAb anti-mouse CD28 (clone 37.51)	Bio X Cell	Cat#BE0015-1
Anti-CD3-PerCP-Cy5.5 (clone 17A2) (1:100)	BD Biosciences	Cat#560527
Anti-NK-1.1-PE (clone PK136) (1:100)	BD Biosciences	Cat#557391
Anti-CD11b-APC-Cy7 (clone M1/70) (1:100)	BD Biosciences	Cat#557657
Anti-CD4-FITC (clone RM4-5) (1:100)	Thermo Fisher Scientific	Cat#11-0042-86
Anti-CD8a-APC (clone 53-6.7) (1:100)	Thermo Fisher Scientific	Cat#17-0081-83
Anti-NKp46-APC (clone 29A1.4) (1:100)	BioLegend	Cat#137608
Anti-CD49b-PE (clone DX5) (1:100)	BioLegend	Cat#108907
Anti-F4/80-FITC (clone Cl:A3-1) (1:100)	CiteAb	Cat#MCA497F
Chemicals, peptides, and recombinant proteins		
DPBS (Ca^{2+} and Mg^{2+}-free)	Thermo Fisher Scientific	Cat#14190094
RPMI-1640 medium, HEPES, no glutamine	Thermo Fisher Scientific	Cat#42401042
MEMα, nucleosides, no phenol red	Thermo Fisher Scientific	Cat#41061029
DMEM/F-12, no glutamine	Thermo Fisher Scientific	Cat#21331020
Fetal bovine serum, qualified, heat inactivated, Brazil	Thermo Fisher Scientific	Cat#10500064
L-glutamine (200 mM)	Thermo Fisher Scientific	Cat#25030081
Penicillin-Streptomycin	Sigma-Aldrich	Cat#P4333-100ML
ACK lysing buffer	Thermo Fisher Scientific	Cat#A1049201
UltraPure 0.5 M EDTA, pH 8.0	Thermo Fisher Scientific	Cat#15575020
2-Mercaptoethanol (55 mM)	Thermo Fisher Scientific	Cat#21985-023
EasySep buffer	STEMCELL Technologies	Cat#20144
Recombinant murine IL-2	PeproTech	Cat#212-12
Recombinant murine IL-15	PeproTech	Cat#210-15
Recombinant murine M-CSF	PeproTech	Cat#315-02
Critical commercial assays		
EasySep Mouse NK Cell Isolation Kit	STEMCELL Technologies	Cat#19855
Experimental models: Organisms/strains		
C57BL/6J, male and female	Janvier Labs	Cat#SC-C57N-F
Other		
6-well suspension plate, non-adherent	Sarstedt	Cat#83.3920.500
Cell culture flask, T-75, surface: suspension, filter cap	Sarstedt	Cat#83.3911.502
Cell culture flask, T-175, surface: suspension, filter cap	Sarstedt	Cat#83.3912.502
Tissue culture flask with re-closable lid 115/150 cm^2	TPP Techno Plastic Products AG	Cat#90552
Centrifuge tubes, conical 50 mL	TPP Techno Plastic Products AG	Cat#91050
Petri dish 100 × 15 mm standard Falcon	Milian SA	Cat#351029
Falcon cell strainers for 50 mL tubes, 40 μm	Milian SA	Cat#352340
Falcon cell strainers for 50 mL tubes, 70 μm	Milian SA	Cat#352350
Falcon cell strainers for 50 mL tubes, 100 μm	Milian SA	Cat#352360

MATERIALS AND EQUIPMENT

Note: Use aseptic techniques to ensure the sterility of all material used under a cell culture hood.

Recommended medium		
Reagent	Final concentration	Amount
DPBS (Ca^{++} and Mg^{++}-free)	N/A	48.9 mL
Fetal Bovine Serum	2%	1 mL
UltraPure 0.5 M EDTA, pH 8.0	1 mM	100 μL
Total	**N/A**	**50 mL**
The solution can be stored for up to 6 months at 4°C.		

DMEM/F12 complete medium		
Reagent	Final concentration	Amount
DMEM/F-12, no glutamine	N/A	500 mL
Fetal Bovine Serum	10%	50 mL
L-Glutamine (200 mM)	2 mM	5 mL
Penicillin-Streptomycin (100×)	1×	5 mL
Total	**N/A**	**560 mL**
The medium can be stored for up to 6 months at 4°C.		

RPMI complete medium		
Reagent	Final concentration	Amount
RPMI-1640 Medium, HEPES, no glutamine	N/A	500 mL
Fetal Bovine Serum	10%	50 mL
L-Glutamine (200 mM)	2 mM	5 mL
Penicillin-Streptomycin (100×)	1×	5 mL
2-mercaptoethanol (55 mM)	0.05 mM	500 μL
Total	**N/A**	**560.5 mL**
The medium can be stored for up to 6 months at 4°C.		

MEMα complete medium		
Reagent	Final concentration	Amount
MEMα, nucleosides, no phenol red	80%	500 mL
Fetal Bovine Serum	20%	100 mL
Penicillin-Streptomycin (100×)	1×	5 mL
2-mercaptoethanol (55 mM)	0.05 mM	550 μL
Total	**N/A**	**605.5 mL**
The medium can be stored for up to 6 months at 4°C.		

Alternatives: STEMCELL Technologies provides the EasySep Buffer (#20144) as an alternative to Recommended Medium.

STEP-BY-STEP METHOD DETAILS

Plate coating with αCD3/αCD28

Timing: 15 min

Plate coating αCD3/αCD28 needs to be performed one day before splenocyte isolation.

1. Dilute both antibodies in 12 mL DPBS to a final concentration of 1 μg/mL for αCD3 and 5 μg/mL for αCD28.
2. Transfer 2 mL of the antibody dilution per well on each well of a 6-well suspension plate and make sure that the wells are fully covered.
3. Wrap the plate in aluminum foil and incubate for 8–16 h at 4°C.

Isolation of splenocytes

Timing: 1 h

The spleen of one C57BL/6 mouse will give ~30–80 × 10^6 splenocytes with ~20%–30% T cells and 1%–2% NK cells.

4. Prepare a 50 mL centrifuge tube with DPBS on ice.
5. Sacrifice one mouse and disinfect the abdominal skin with 70% ethanol.
6. Isolate the spleen with sterile instruments.
   a. Open the peritoneum using a forceps and scissors.
   b. Expose the spleen by pulling associated white connective tissue with forceps.
   c. Remove unwanted tissue with scissors and transfer the spleen into the 50 mL centrifuge tube.

Pause point: The spleen can be kept in DPBS on ice for transport. Try to process within 2 h after isolation.

7. Place a 100 μm cell strainer in a new 50 mL centrifuge tube and mash the spleen with a plunger of a 2 mL syringe. Continuously moisten the cell strainer with DPBS while mashing. Fill the tube with DPBS.

⚠ CRITICAL: Ensure that the tissue stays moistened with DPBS throughout this step.

8. Spin at 400 g for 5 min and aspirate the supernatant.
9. Resuspend the red pellet in room tempered ACK lysis buffer using a 1000 μL pipette.
   a. Use 500 μL ACK lysis buffer/spleen.
   b. Incubate for 2–3 min and fill the tube with DPBS.
10. Pass cells through a 40 or 70 μm cell strainer to remove cell clumps and debris. Moisten the cell strainer with DPBS or medium before use.
11. Spin at 400 g for 5 min and aspirate the supernatant.
12. Resuspend the pellet in RPMI complete medium (for T cell expansion) or recommended medium (for NK cell expansion) to get ~8–12 × 10^6 cells/mL.
13. Count cells with a hemocytometer at a 1:20 dilution.

Note: Proceed with T cell or NK cell expansion after this step.

T cell expansion

Timing: 5–7 days

Splenocytes will be activated with αCD3/αCD28 antibodies for 2 days and further expanded with IL-2. During activation, T cells will form clusters and will increase in size. After 3–5 days, an increased proliferation rate, an elongated shape and clonal clusters can be observed.

⚠ CRITICAL: Ensure you have splenocytes counted and ready for seeding before starting.

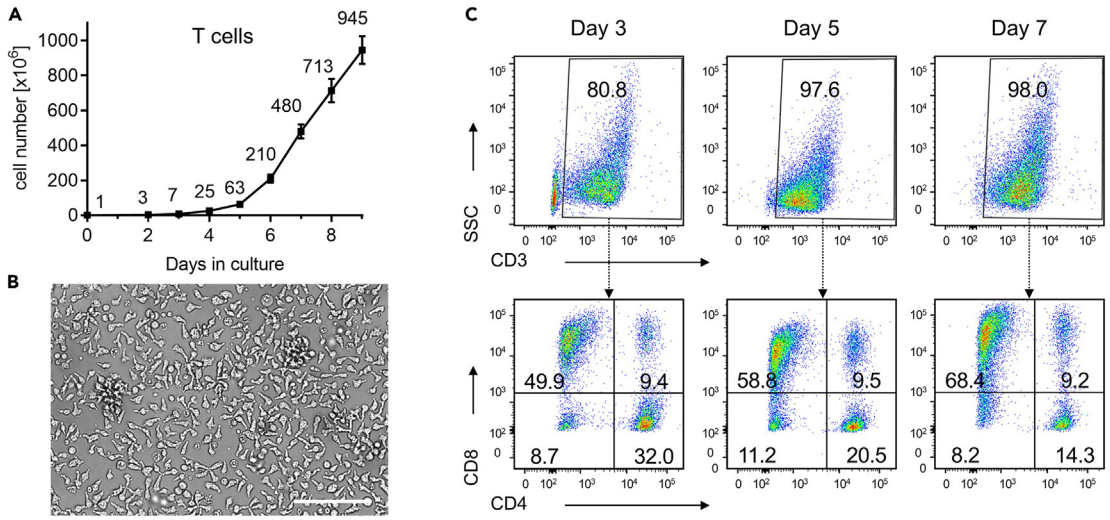

Figure 1. T cell proliferation and phenotypic characterization during expansion

(A) Expansion of T cells over time starting from 1 × 10^6 splenocytes. Error bars are based on n = 3 replicates. Mean cell numbers (in ×10^6) are indicated for each measurement time point.

(B) Microscopic image of T cells on day 6 of expansion. As expected, T cells show elongated shape and clonal clusters indicative for a proliferative state. Scale bar is 100 μm.

(C) Flow cytometry of $CD3^+$ T cells on day 3, 5 and 7 of expansion including the relative proportion of $CD4^+$ and $CD8^+$ T cells. SSC, side scatter.

14. Aspirate the remaining supernatant of the αCD3/CD28-coated 6-well suspension plates. Wash one time with DPBS to remove unbound antibodies and repeat aspiration.
15. Seed 5 × 10^6 isolated splenocytes in 5 mL RPMI complete medium per coated well.
16. On day 1, supplement the medium with 50 U/mL IL-2.
17. On day 2, harvest cells with a serological pipette.
    a. Detach the cells by pipetting up and down with a serological pipette and transfer the cell suspension into a 50 mL centrifuge tube.
    b. Add 1 mL DPBS to remaining cells and detach with a 1000 μL pipette. Combine cell fractions.
    c. Spin the cell suspension at 400 g for 5 min, resuspend in appropriate volume of RPMI complete medium and count. Reseed cells at a concentration of 1 × 10^6 cells/mL with 0.5 mL/cm^2. Supplement the medium with 50 U/mL IL-2.

Note: Activated T cells can be easily detached by resuspension.

18. From now on, count cell numbers every 1–2 days and expand with additional RPMI complete medium to maintain a concentration of 1 × 10^6 cells/mL with 0.5 mL/cm^2. Supplement additional medium with 50 U/mL IL-2.
19. T cells show >95% purity and high viability 5–7 days after isolation (Figure 1) and can be used for *in vitro* and *in vivo* experiments.

Note: For long-term culture and higher number of cells increase IL-2 dose to 200 U/mL from day 6 onward. With increased IL-2 concentration T cells can be cultured for up to 11 days.

NK cell expansion

Timing: 7–12 days

The following steps are written for the processing of splenocytes from two C57BL/6 mouse spleens of which ~1.5 × 10^6 NK cells can be isolated. NK cells show an increased proliferation rate after 3–5 days.

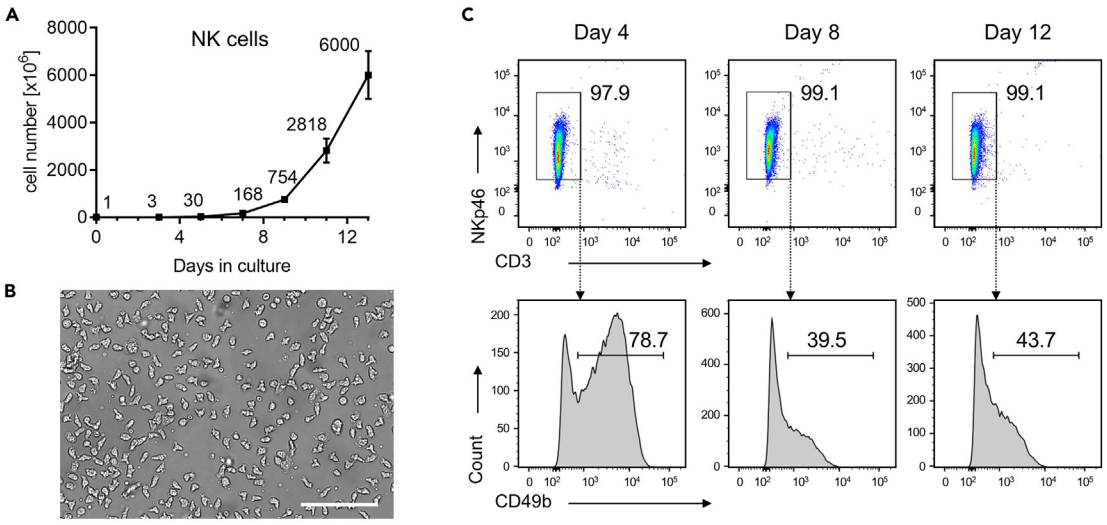

Figure 2. NK cell proliferation and phenotypic characterization during expansion

(A) Expansion of NK cells over time starting from 1 × 10^6 splenocytes. Error bars are based on $n = 3$ replicates. Mean cell numbers (in × 10^6) are indicated for each measurement time point.

(B) Microscopic image of NK cells on day 8 of expansion. As expected, NK cells show elongated shape indicative for a proliferative state. Scale bar is 100 μm.

(C) Flow cytometry of $CD3^-NKp46^+$ NK cells on day 4, 8 and 12 of expansion including the relative expression CD49b expression.

⚠ CRITICAL: Ensure you have splenocytes counted and ready for seeding before starting. Cell concentration and medium volume are important. Deviations will hamper proliferation and viability.

20. Spin splenocytes in recommended medium at 400 g for 5 min and aspirate the supernatant.
21. Resuspend the splenocyte pellet at 1 × 10^6 cells/mL in recommended medium and follow the instructions of the EasySep Mouse NK Cell Isolation Kit.
22. Spin the isolated NK cell suspension at 400 g for 5 min and aspirate the supernatant.
23. Resuspend the pellet in 1 mL medium to get ~1–2 × 10^6 cells/mL.
24. Count cells with a hemocytometer at a 1:5 dilution.
25. Seed NK cells at a concentration of 0.5 × 10^6 cells/mL in 800 μL MEMα complete medium/well on a 24-well suspension plate. Supplement medium with 150 ng/mL IL-15.
26. On day 3, count the cell suspension with a hemocytometer and expand with additional MEMα complete medium to reach 0.4 × 10^6 cells/mL with 0.25 mL/cm^2. Supplement additional medium with 150 ng/mL IL-15.
27. From now on, count cell numbers every 2 days and expand with additional MEMα complete medium to maintain a concentration of 0.4 × 10^6 cells/mL with 0.25 mL/cm^2. Supplement additional medium with 150 ng/mL IL-15.
28. NK cells show >95% purity 4 days after isolation (Figure 2). High viability is maintained for 12 days after isolation. NK cells are recommended to be used 7–12 days after isolation for *in vitro* and *in vivo* experiments.

⚠ CRITICAL: Keep NK cells on uncoated suspension plates/flasks.

Note: The viability of NK cells will reduce after 1 day if not stimulated or deprived from IL-15.

Isolation of bone marrow cells

Timing: 1.5 h

Both femora and tibiae of one C57BL/6 mouse will give ~50–90 × 10^6 bone marrow cells.

29. Prepare a 50 mL centrifuge tube with DPBS on ice.
30. Sacrifice one mouse and disinfect the skin at the legs and abdomen with 70% ethanol.
31. Make an incision at the top of each hind leg and pull the skin down towards the foot to expose the muscle. Cut the cartilage between femur and tibia.
32. Use scissors to free the hind legs from muscles and perform the amputation.

Note: Twisting will help to isolate the femur. But be careful not to break the bones.

33. Use a paper tissue sprayed with 70% ethanol to remove remaining muscle tissue from the bones.
34. Spray the bones with 70% ethanol to disinfect and wait 30 s. Transfer them into the 50 mL centrifuge tube filled with DPBS for transport.

Pause point: The bones can be kept in DPBS on ice for transport. Try to process within 2 h after isolation.

35. Place the bones on a petri dish under a sterile working bench. Remove remaining connective tissue with forceps and scalpel by scratching. Cut the cartilage at both sides of the bone to allow excess to the bone marrow.

⚠ CRITICAL: Process bones of max. 2 mice at the same time to not let the bone marrow dry out.

36. Attach a 25G needle to a 10 mL syringe. Flush the bone cavity with DMEM/F12 complete medium into a 50 mL centrifuge tube until the bone cavity appears white.
37. Spin at 400 g for 5 min and aspirate the supernatant.
38. Resuspend the red pellet in room tempered ACK lysis buffer using a 1000 µL pipette.
    a. Use 500 µL ACK lysis buffer/spleen.
    b. Incubate for 2–3 min and fill with DPBS.
39. Pass the cells through a 40 µm or 70 µm cell strainer to remove cell clumps and debris. Moisten the cell strainer with DPBS or medium before use.
40. Spin at 400 g for 5 min and aspirate the supernatant.
41. Resuspend the pellet in DMEM/F12 complete medium to get ~4–6 × 10^6 cells/mL.
42. Count the bone marrow cells with an automated cell counter or a hemocytometer at a 1:10 dilution.

Note: Proceed with BMDM differentiation and expansion after this step.

BMDM differentiation and expansion

Timing: 5–7 days

On day 1–3 of differentiation dead floating cells can be observed. Adherent macrophages will become visible on day 3–4 of differentiation. Each 10 cm Petri dish will give ~8 × 10^6 BMDMs on day 5 and ~18 × 10^6 BMDMs on day 7.

⚠ CRITICAL: Ensure you have bone marrow cells counted and ready for seeding before starting.

43. Dilute bone marrow cells to a concentration of 0.2–0.25 × 10^6 cells/mL in 12 mL DMEM/F-12 complete medium supplemented with 20% L929 conditioned medium and seed 12 mL cell suspension per non-adherent 10 cm petri dish.

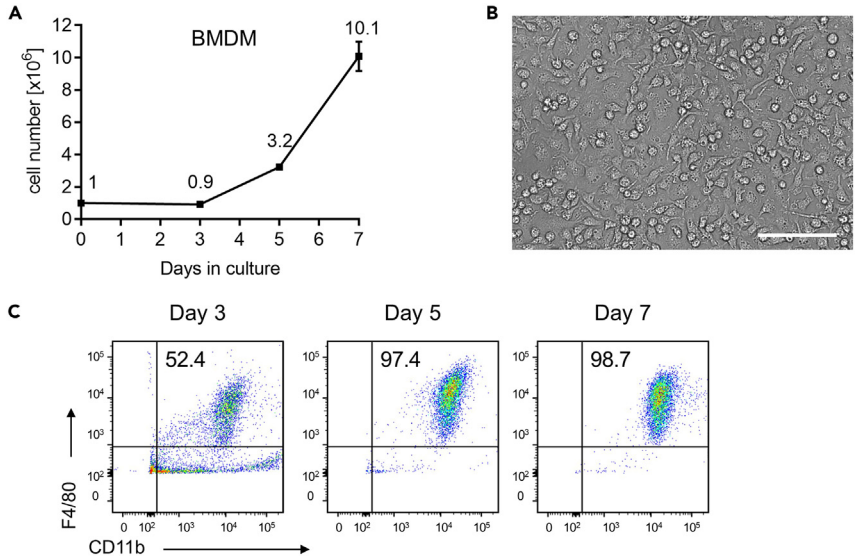

Figure 3. BMDM proliferation and phenotypic characterization during expansion

(A) Expansion of BMDMs over time starting from 1 × 10^6 bone marrow cells. Error bars are based on n = 3 replicates. Mean cell numbers (in ×10^6) are indicated for each measurement time point.

(B) Microscopic image of BMDMs on day 5 of expansion. As expected, cells are attached to the plate indicative for a successful differentiation of bone marrow cells to BMDMs. Scale bar is 100 μm.

(C) Flow cytometry of $CD11b^+F4/80^+$ BMDMs on day 3, 5 and 7 of expansion.

Note: Alternative to 20% L929 conditioned medium also 20 ng/mL mouse M-CSF can be added to the medium.

44. Perform partial medium change on day 3 and day 5. For long-term culture, repeat every other day.
    a. Carefully tilt the plate and gently remove 7 mL medium with a serological pipette.
    b. Replace with fresh DMEM/F-12 complete medium supplemented with 20% L929 conditioned medium to again reach a total volume of 12 mL.

⚠ CRITICAL: Differentiating BMDMs are not firmly attached on day 3. Be careful to aspirate medium from the top.

45. After 5–7 days, aspirate the medium from the plate. Gently wash the cells 1× with 5 mL DPBS.
46. Detach macrophages using cold DPBS with 2.5 mM EDTA:
    a. Add 5 mL of cold DPBS with 2.5 mM EDTA per plate.
    b. Incubate for 10–20 min on ice or at 4°C in the fridge.
    c. Gently resuspend detaching cells with a serological pipette and collect them in a 50 mL centrifuge tube. Add additional DPBS + 2.5 mM EDTA to collect remaining cells.
47. Centrifuge at 350 g for 5 min and discard the supernatant.
48. Flick the pellet and resuspend the cells in required buffer or medium.
49. BMDMs show >95% purity 5 days after isolation (Figure 3). They might be kept in culture for up to 2 weeks but will become more differentiated and adherent with time. With respect to the seeding concentration in this protocol, BMDMs are recommended to be used 5–7 days after isolation for *in vitro* and *in vivo* experiments.

EXPECTED OUTCOMES

The present protocol has been validated in multiple differentiation experiments and cell expansion numbers and phenotypic characteristics remained stable (Figures 1, 2, and 3). However, we recommend confirming cell phenotypes when the protocol is established.

Expanded immune effector cells can be used for a variety of *in vitro* and *in vivo* studies. These can be immunological *in vitro* studies to investigate immune cell interactions in co-culture experiments as well as *in vitro* studies to analyze changes in phenotype and intracellular signaling after stimulation or inhibition. In a pre-clinical setting, the high number expansion allows to use the immune effector cells with or without modification for adoptive cell transfer *in vivo*. Studies using the same protocol for the generation of mRNA-based chimeric antigen receptor (CAR) expressing mouse T cells and the investigation of the effect of immunocytokines on T cell killing have already been published.[5,6] A study comparing all three immune effector cells transfected with mRNA coding for a CAR is ongoing.

Overall, this manuscript provides protocols for the rapid and high number expansion of mouse T cells, NK cells and BMDMs and serves as a valuable template to better standardize their phenotype on the day of use.

LIMITATIONS

These protocols are referring to organ harvesting of C57BL/6 mice. The total numbers of splenocytes and bone marrow cells that can be isolated might be reduced if other mouse strains are used. Variations might also occur depending on the age of mice as splenocyte numbers decrease from 3- to 5-months old mice.[7] Therefore, for harvesting a maximal splenocyte number, 3-months old mice are recommended. Processing times should be held short to allow maximal expansion of cells.

One has to consider that the protocol aims to standardize the phenotypical state of the immune effector cells on the day of use. However, after long-term expansion it does not reflect every phenotypical state of T cells, NK cells and BMDMs *in vivo* as it is influenced by many factors such as development stage, tissue context and physiological condition of the animal. The usability of long-term expanded immune effector cells for own studies should be evaluated before conducting them. Otherwise, further adaptions need to be investigated such as complex cell culture, different culture media and the supplementation with additional cytokines.

TROUBLESHOOTING

Problem 1

Low number of isolated splenocytes, related to step 6–7.

Potential solution

- Ensure the maintenance of spleen tissue integrity and remove surrounding connective tissue before transfer of spleens to DPBS.
- Reduce transport times of spleens in DPBS.
- Use a maximum of 2 spleens per cell strainer.
- Ensure that the cell strainer is moistened when passing the splenocytes.

Problem 2

T cells are not activated and do not increase in size, related to steps 14–17.

Potential solution

- Ensure that you used the right concentration of αCD3 and αCD28 antibodies for coating.
- Make sure that you properly wash the αCD3/αCD28-coated plates before adding the cell suspension.
- Ensure that the viability of splenocytes was high at the day of isolation.
- Make sure that you added IL-2 on day 1 after seeding.

Problem 3

T cells do not expand well, related to steps 18–19.

Potential solution

- Control the cell concentration and medium volume throughout the expansion.
- Store IL-2 at −80°C and only keep it at 4°C for a maximum of 2 weeks.
- Ensure that you supplement additional medium with 50 U/mL IL-2 for each expansion.
- Make sure that the RPMI complete medium contains all listed supplements.

Problem 4

Low number of isolated NK cells from splenocytes, related to steps 6–7 and steps 20–21.

Potential solution

- Ensure the maintenance of spleen tissue integrity and remove surrounding connective tissue before transfer of spleens to DPBS.
- Reduce transport times of spleens in DPBS.
- Ensure that the cell strainer is moistened when passing the splenocytes.
- Pay regard to the instructions and incubation times of the EasySep Mouse NK Cell Isolation Kit.

Problem 5

NK cells do not expand well, related to steps 25–28.

Potential solution

- Control the cell concentration and medium volume throughout the expansion.
- Store IL-15 at −80°C and only keep it at 4°C for a maximum of 2 weeks.
- Ensure that you supplement additional medium with 150 ng/mL IL-15 for each expansion.
- Make sure that the MEMα complete medium contains all listed supplements.

Problem 6

NK cells attach to plates/flask, related to step 28.

Potential solution

- Make sure that you use suspension plates/flasks for NK cell expansion.
- Flush with cold DPBS to detach NK cells.

Problem 7

Low number of isolated bone marrow cells, related to steps 34–36.

Potential solution

- Ensure the maintenance of bone integrity and remove surrounding muscle tissue before transfer of bones to DPBS.
- Do not exceed sterilization time of bones with 70% ethanol.
- Reduce transport times of bones in DPBS.
- Reduce scratching times or process less bones at the same time.
- Flush bones thoroughly until the cavity appears white.

Problem 8

BMDMs do not expand well, related to steps 43–44.

Potential solution

- Control the cell concentration and medium volume throughout the expansion.
- Be careful not to aspirate bone marrow cells during the partial medium change on day 3.
- Store L929 conditioned medium at −80°C.
- Ensure that you supplement DMEM/F12 complete medium with 20% L929 conditioned medium for each expansion.
- Make sure that the DMEM/F12 complete medium contains all listed supplements.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Tobias Weiss (tobias.weiss@usz.ch).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This study did not generate/analyze datasets/code.

ACKNOWLEDGMENTS

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L929 cells were kindly provided by Dr. Tomasz Rygiel at the Department of Immunology, Medical University of Warsaw.

AUTHOR CONTRIBUTIONS

T.L. established the NK cell and BMDM expansion protocols and generated and analyzed the data. H.M. established the T cell expansion protocol. T.W. overlooked the study. T.L., T.W., and M.W. wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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