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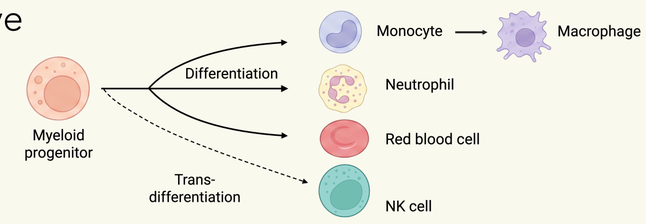
In Vitro NK-Cell Transdifferentiation from Murine Myeloid Progenitors

INTRODUCTION

- **Natural Killer (NK)** cells, part of the innate immune system, perform **immunosurveillance** to eliminate virus-infected and cancerous cells[1]
- Their anti-tumoral properties are being explored as a promising **cancer immunotherapy**, yet greater research into NK cells is required
- **Hoxb8-FL cell lines** are rapidly proliferating, immortalized murine hematopoietic progenitor cells (**HPCs**), that provide an ideal model for differentiation and immune cell studies[2]
- While Hoxb8-FL have thought to differentiate **exclusively along myeloid lineages**, *human* equivalent HPCs (CD34⁺) have **transdifferentiated down lymphoid lineages,** to produce NK[3]



- No *murine* NK-transdifferentiation protocol exists, yet it would allow for a reliable, rapid NK source and deepen understandings of NK-development, aiding further therapeutic uses

RESEARCH AIMS

- **Primary aim:** obtain NK cells from the murine myeloid progenitor cell line, Hoxb8-FL
- **Secondary aim:** culture macrophage, following the natural myeloid lineage of Hoxb8-FL

RESULTS

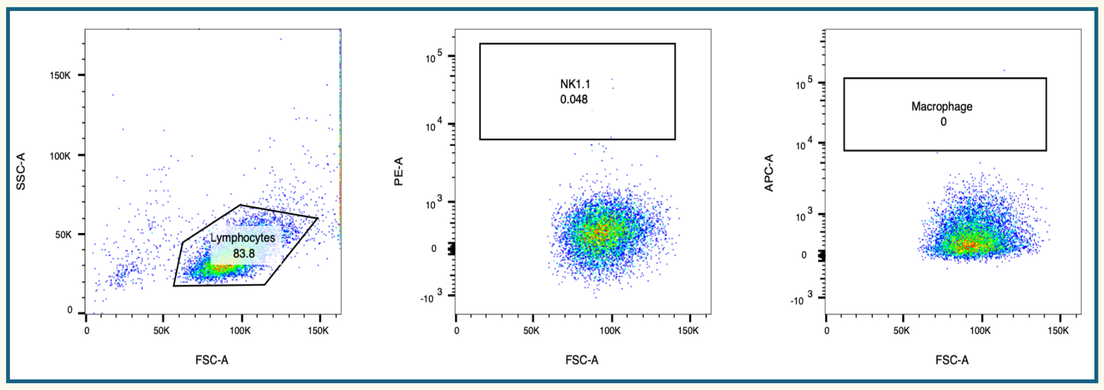


Figure 1 – Flow Cytometry of Control Medium 1

Of **83.8% lymphocytes**, after single and live cell gating:

- **0.048% NK 1.1⁺**
- **0% F4/80⁺**

This suggests neither NK, nor macrophage differentiated under these conditions

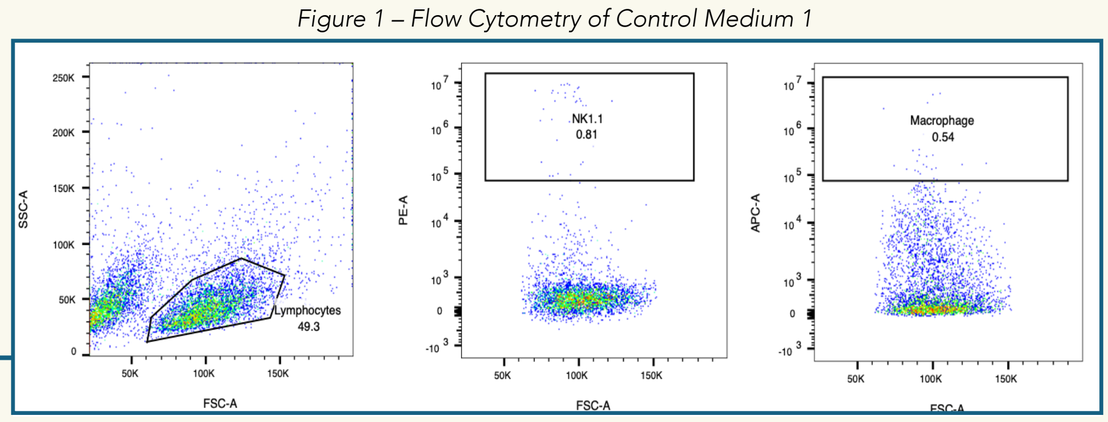


Figure 2 – Flow Cytometry of Culture Medium 5

Of **49.3% lymphocytes**, after single and live cell gating:

- **0.81% NK 1.1⁺**
- **0.54% F4/80⁺**

This suggests NK cell-transdifferentiation occurred while macrophage-differentiation remained low

METHOD

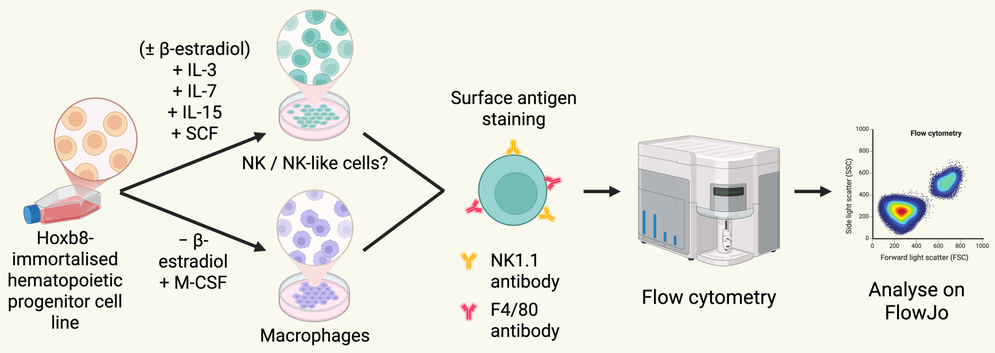
CONTROL MEDIUMS

- **Control Medium 1** – Negative Control: undifferentiated WT Hoxb8-FL
  - RPMI 1640 + 10% FBS + 1% PSQ + 0.1% **β**-ME (STANDARD) + 70 ng/mL FLT3L + **272ng/mL β-estradiol**
- **Control Medium 3** – Positive control: macrophage differentiation
  - STANDARD + FLT3L + **50 ng/mL M-CSF**

CULTURE MEDIUMS

- **Culture Medium 5** (CM5)**:** all cytokines and low **β**-estradiol
  - STANDARD + FLT3L + **30 ng/mL β-estradiol + IL-3 (5) + IL-7 (20) + IL-15 (10) + rhSCF (20)** (all conc. ng/mL)

All cultures were routinely passaged, incubated at 37°C with 5% CO_2



FLOW CYTOMETRY PREPARATIONS

- Sample were collected and resuspended in 5% FBS/PBS
- **Antibody staining:** NK 1.1 (PE) + F4/80 (APC), incubated 30 mins at 4°C dark, washed PBS
- **Viability staining:** DAPI (1:1000)

Flow cytometry and data analysis: BD FACSymphony A1 drop plots analysed using FlowJo v10.8.1 *(Figures 1 & 2)*

MACROPHAGE STAINING

- **Seeded and fixed:** 3x10^4 per well, 4% PFA
- **Permeablisied and blocked**: 0.1% Triton X-100, 5% BSA
- **Primary and Secondary antibody:** F4/80 (1:100), Goat anti-Rat IgG (H+L) (1:1000)
- **DAPI staining:** DAPI (1:1000)

Incubation and washing (various conditions) between each stage *(Figure 4)*

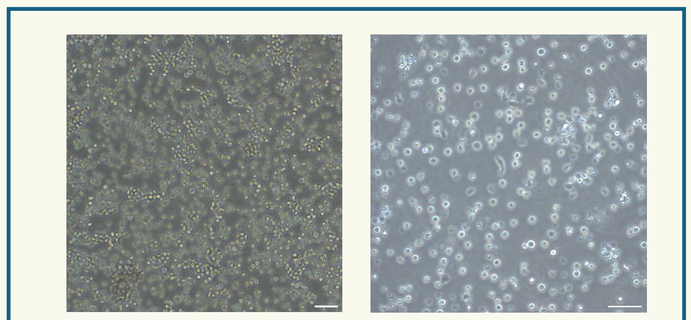


Figure 3 – Microscopy images. LEFT: Control.M 1 - day 3 (Scale bar = 200μm) RIGHT: Culture.M 5 - day 11 (Scale bar = 50μm).

- **WT Hoxb8 cells** remained stable and healthy at day 3 (Left)
- **Culture Medium 5** (Right) at day 11 showed **morphological differences**, yet reduced cell health

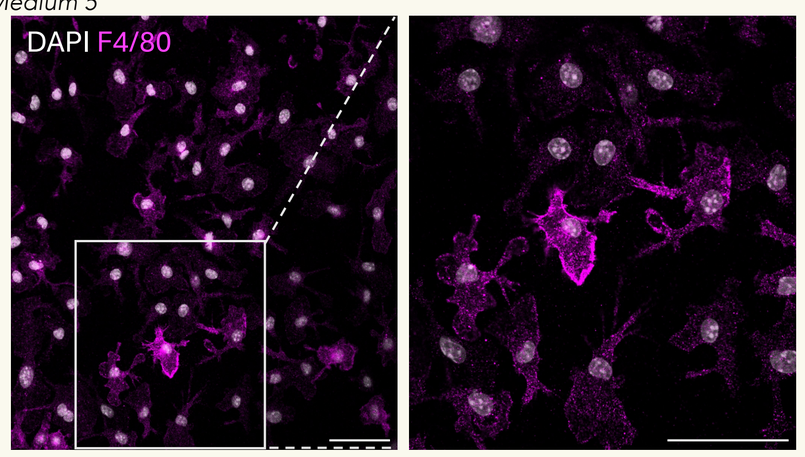


Figure 4 - Immunofluorescence staining Control Medium 3 (M-CSF): F4/80 (magenta) identifies macrophage. DAPI (white) identifies nuclei (Scale bar = 50μm)

- F4/80 staining confirmed **macrophage** presence, shown by magenta fluorescence
- Cell morphology had irregular and extended membranes, expected of macrophage

DISCUSSION

- **FLT3L** supported cell survival. Reducing it caused high cell death, which decreased when restored to **70ng/mL**.
- **β-estradiol** supported Hoxb8-FL maintenance. Removal reduced viability (35.5–52.2%) compared to cultures containing it (e.g **75.5% in CM5**).
- **Macrophage differentiation** was highly successful (**CM3 had 93.9%** F4/80⁺ cells), reflecting Hoxb8's natural myeloid pathway.
- **NK 1.1⁺** expression **increased** from basal level in CM5, yet remained low likely due to the difficulty of transdifferentiation.
- **CM5**, that retained Hoxb8 survival conditions, produced the highest NK 1.1⁺ population suggesting cell survival is important for successful NK transdifferentiation.

CONCLUSION

- Maintaining **Hoxb8-FL survival conditions may be essential** to allow sufficient time for NK-cell transdifferentiation
- **Culture Medium 5 had the largest increase of NK 1.1⁺** cells, implying survival conditions and cytokines aid transdifferentiation

FUTURE RESEARCH

- Extend culture durations (e.g 2,3 & 4 weeks), analysing weekly with Flow Cytometry, to investigate if higher proportions of NK 1.1⁺ can be achieved, as reported by Grzywacz et al. (2011) with CD34⁺.[3]

REFERENCES

1) Thermofisher.com. (2016). *Natural Killer Cells Overview*
2) Redecke, V. et al. (2013). *Hematopoietic Progenitor Cell Lines with Myeloid and Lymphoid Potential*. Nature Methods. 10(8), 795–803.
3) Grzywacz, B et al. (2011). *Natural killer–cell differentiation by myeloid progenitors.* Blood. 117(13), 3548–3558.