ANIMAL TISSUES

ISOLATION AND IDENTIFICATION OF VIABLE IMMUNE CELLS FROM MURINE LUNGS

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CONTEXT

This research focuses on the immune response during lung inflammation and/or infection *in vivo*.

This requires isolating viable cells from lungs and characterising the immune cell populations by flow cytometry.

Our standard protocol for isolating viable cells from lung tissue involves creating a single cell suspension by passing the tissue through a 70 µm cell sieve, which, when working with multiple samples, is highly time-consuming.

Here we describe a novel rapid protocol for the isolation of immune cells from mice lungs using the Precellys Evolution tissue homogeniser.

MATERIALS

Instrument: Precellys® Evolution

Lysing kit: 7 mL CK28 (Cat no KT03961-1-302.7)

Sample: Mouse whole lung (~0.3 g; fresh)

Buffer: Sterile endotoxin-free PBS (1 mL per tube)

PROTOCOL

1) Collect the whole lung directly in the Precellys® tube (or a bijou for the sieve hand grinding method) containing 1 ml PBS

2) Beat the organ in the Precellys® for 1 cycle of 10 s at 4500 rpm (RT)

3) Filter the homogenate with a 70 µm strainer to remove big debris and beads, rinse with an additional 1 mL PBS

[OR 2'-3') Push the lung through a 70 µm sieve using the plunger of a 5 ml syringe and rinse with 1 mL of PBS]

4) Centrifuge at 600 g for 10 min

5) Lyse the red blood cells (ACK buffer or alternative method)

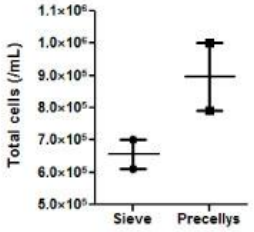
6) After centrifugation, resuspend the cells in 0.5 ml PBS

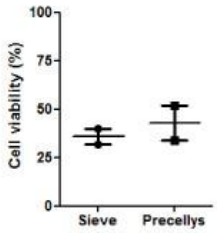
7) Count the total and viable cells using trypan blue exclusion

8) Stain the cells for flow cytometry (here we used antibodies against CD45, CD11b, GR1, F4/80 and CD3).

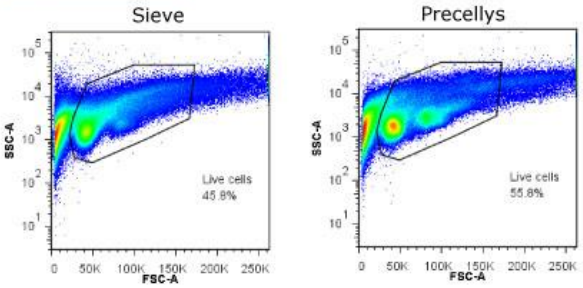
RESULTS

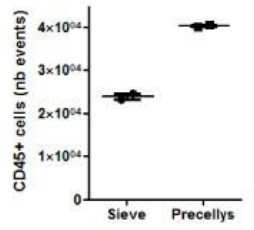
Cell count and viability (Trypan blue)

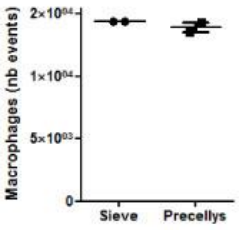


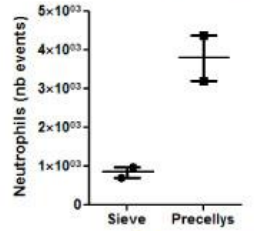


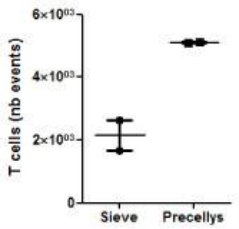
Flow cytometry











CUSTOMER

Queen's University Belfast

Dr Alice Dubois
(Dr Rebecca Ingram's lab)

CONCLUSION

The optimised conditions described here allow the Precellys® Evolution, combined with the dedicated lysing kit, to be utilised for the isolation of viable immune cells from murine lungs that are suitable for phenotypic characterisation. While the yield of macrophages recovery is comparable to a standard manual method, the Precellys® Evolution leads to a higher yield of some immune cells (CD45+), notably neutrophils and lymphocytes.

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