Arising *bona fide* macrophages and dendritic cells from bovine bone marrow

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Introduction

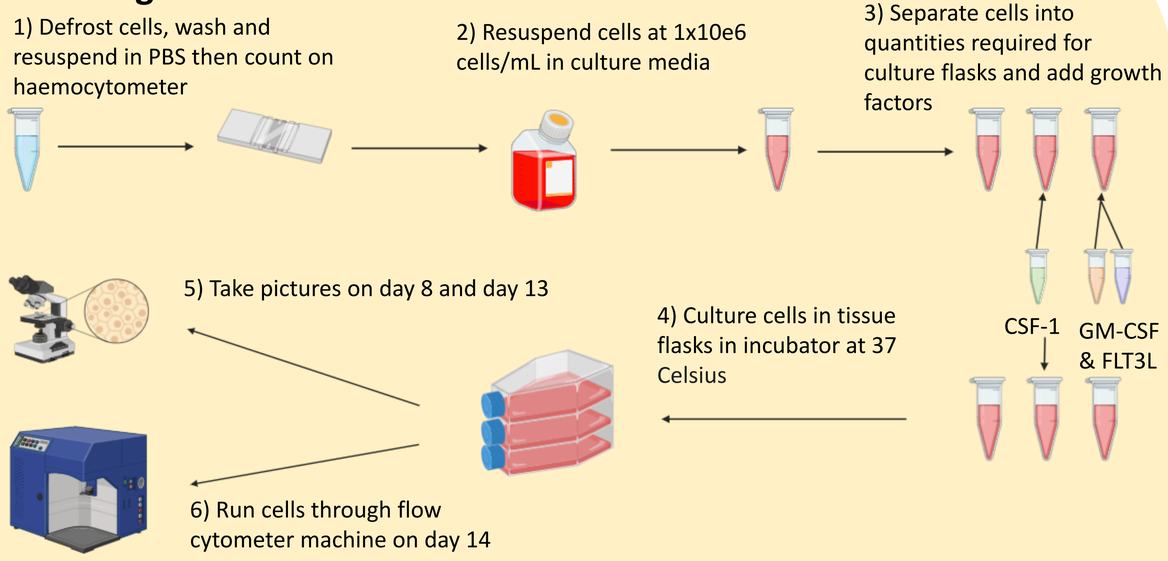
- Arousal of *bona fide* dendritic cells (DC) and macrophages (MO) in humans and mouse models is well defined, however, this is not the case in bovine samples. As an important model for *Mycobacterium infections*, establishing a protocol for arising DC and MO from cattle bone marrow is crucial.

Aims

- To arise DC and MO from primary cattle bone marrow samples using bovine cytokines (GM-CSF and FLT3L for DC CSF-1 or MO).
- To characterise arisen cells phenotypically and by the expression of relevant cell surface markers to validate identity.

Methods

Culturing



Assaying

- Disassociate cells from flasks with Cell Disassociation Solution Non-enzymatic and incubate for 5 minutes.
- Wash cells and count on haemocytometer.
- Resuspend cells at 5x10e5 cells/mL with blocking buffer into separate flasks.
- Incubate for 20 mins.
- Wash cells and resuspend in PBS with relevant antibodies.
- Incubate for 60 mins.
- Wash cells three times, resuspend in PBS and run through FASCs machine.

Results

Fig. 1: Progression of bone marrow cell development over time. **A)** Control cells, no cytokines. *1*: control cells day 8; *2*: control cells day 13 **B)** Cells with GM-CSF and FLT3L added. *1:* cells with GM-CSF and FLT3L at day 8 8; *2:* cells with GM-CSF and FLT3L day 13. **C)** Cells with CSF-1 added. *1:* cells with CSF-1 at day 8; *2:* cells with CSF-1 day 13.

Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; FLT3L, Fms-related tyrosine kinase 3 ligand; CSF-1, colony-stimulating factor 1

Results

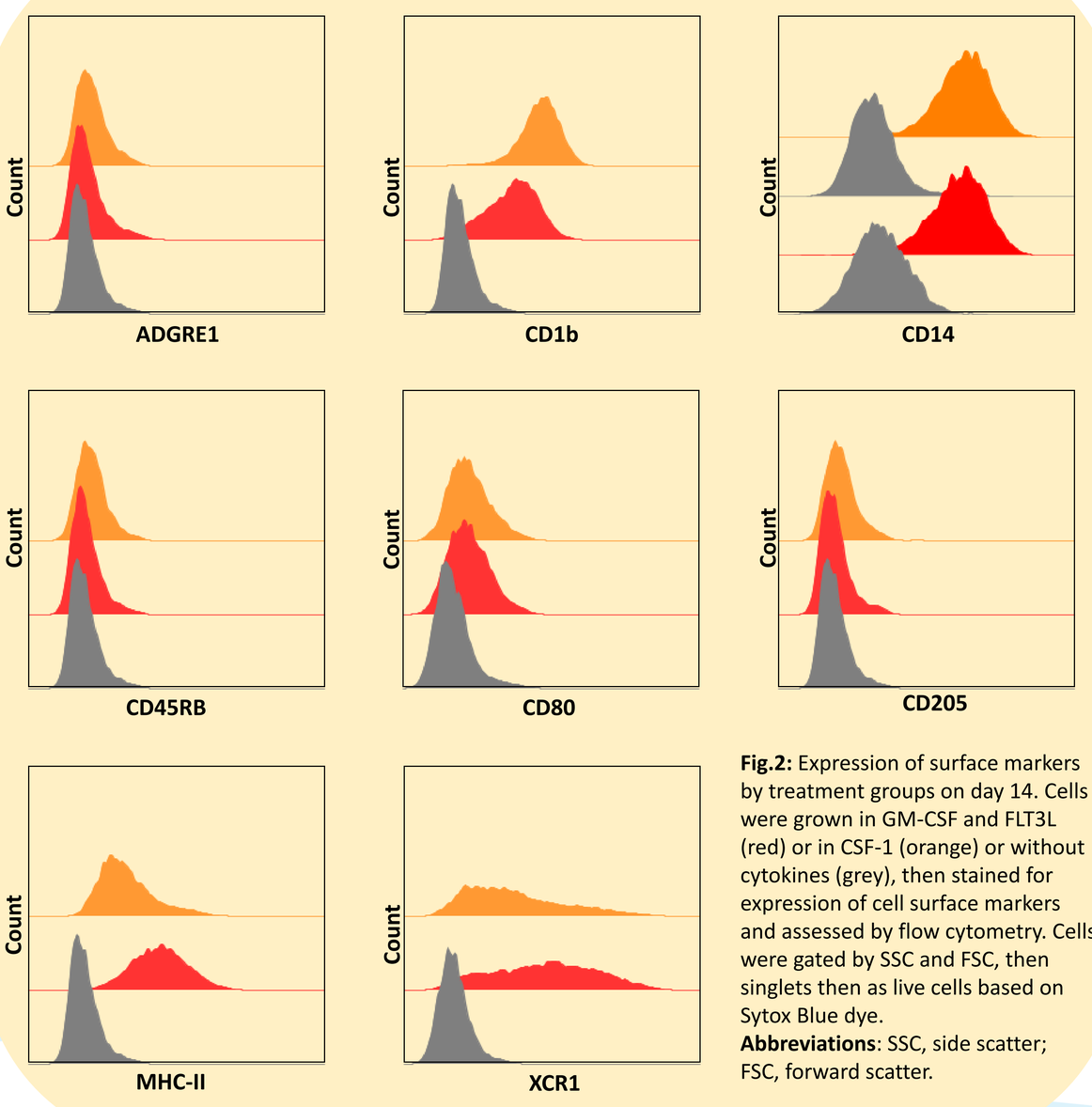


Fig.2: Expression of surface markers by treatment groups on day 14. Cells were grown in GM-CSF and FLT3L (red) or in CSF-1 (orange) or without cytokines (grey), then stained for expression of cell surface markers and assessed by flow cytometry. Cells were gated by SSC and FSC, then singlets then as live cells based on Sytox Blue dye.
Abbreviations: SSC, side scatter; FSC, forward scatter.

Conclusions

- The use of GM-CSF & FLT3L and CSF-1 does produce two distinct cell populations.
- These populations can be identified as macrophages and dendritic cells based on expression of molecules and features known to be associated with these cell types in other species from the literature.

Dendritic cells

- Medium adhesion to cell surface
- Formation of lamellipodia and extensions on cell surface
- Circular cell shape
- High level of MHC-II and XCR1 on cell surface.

Macrophages

- Strong adhesion to flask surface
- Large, irregular cell size
- Cell shape reminiscent of fried egg
- Presence of CD1b and CD14 on cell surface.
- Lower levels of MHC-II and XCR1 on the cell surface.

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