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The Immunological Toolbox: Expanding the portfolio of veterinary immunology reagents

BACKGROUND & REMIT:

- The study of veterinary immunology is currently limited by the availability of species specific reagents.
- The Immunological Toolbox aims to expand the veterinary reagent portfolio through the production and characterisation of immunological reagents, developing new tools, reagents and assays.
- The Roslin Institute Immunological Toolbox is a BBSRC core strategic funded facility which aims to expand the available portfolio through the production and characterisation of novel immunological reagents. This initiative focuses on the development of new recombinant proteins, monoclonal antibodies (mAbs), assays and tools which will allow the advancement of understanding in the field of veterinary immunology; broadening the potential for research projects that are currently restricted by the lack of available reagents.
- The Immunological Toolbox at The Roslin Institute is closely linked with related activity at the Pirbright Institute. At the Pirbright Institute the Toolbox activities include sequencing of mAbs and production of recombinant antibodies.
- At The Roslin Institute we have already produced a number of novel mAbs and recombinant proteins which we aim to commercialise and make available to the wider community. Some key examples are shown here.

MONOCLONAL ANTIBODY PRODUCTION:

Mouse anti-porcine ADGRE1 mAb production

- ADGRE1 is a monocyte/macrophage differentiation marker, an alternative to the well-recognised rodent F4/80.
- Mouse anti-porcine mAbs were produced by immunising mice with ADGRE1 recombinant protein, & optimised by flow cytometry and IHC[1]

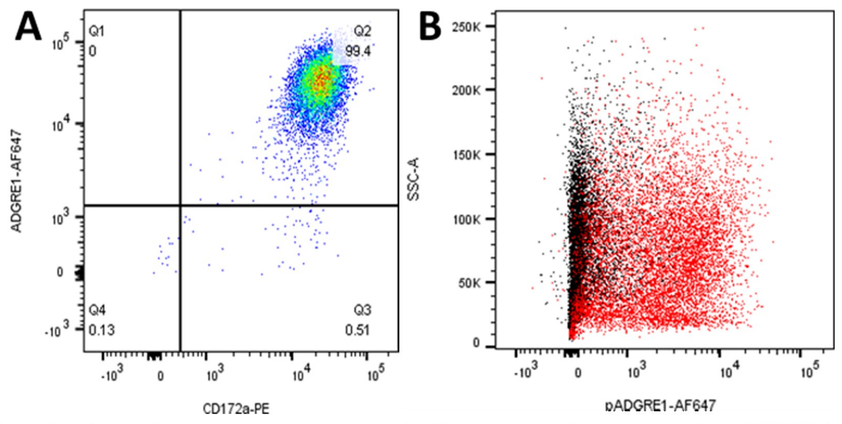

Figure 1. Flow cytometry on pig alveolar macrophages and day 7 bone marrow derived macrophages (BMDMs). ADGRE1 is highly expressed on the cell surface of macrophages from bronchoalveolar lavage as identified by their co-expression of anti-pig CD172a (A). BMDMs after 7 days cultured in rhCSF1 are uniformly ADGRE1+ (red) as compared to an isotype control (black). Exclusion of dead cells using Sytox blue.

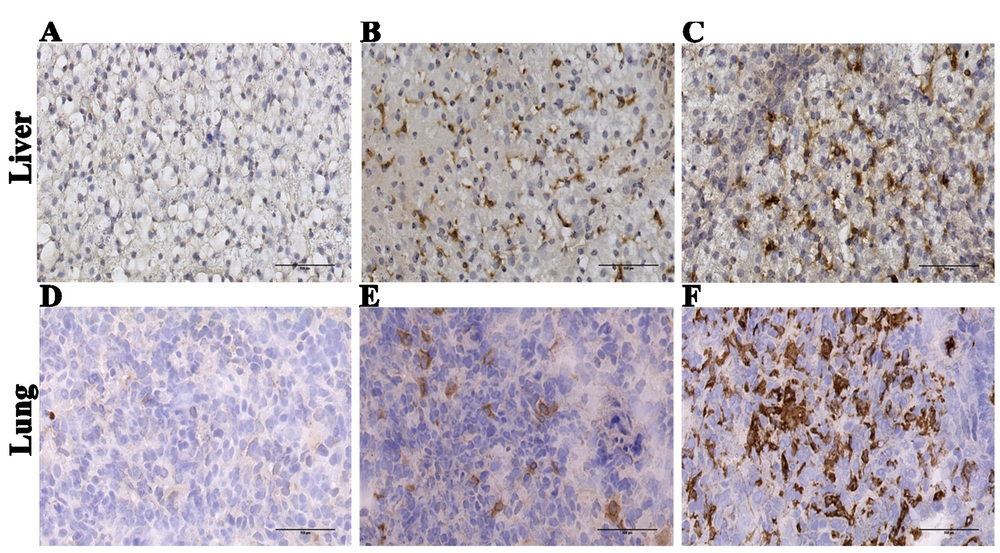

Figure 2 . Immunohistochemical localisation of ADGRE1 in pig tissues. Immunohistochemical staining was carried out on frozen pig liver (A-C) and lung (D-F) tissue. Representative images are shown (n = 3). Tissues were incubated without primary antibody (negative control A, D), antibody recognising CD163 (positive control B, E) and mAb recognising ADGRE1 (C, F). Scale bar =100 µm.

ASSAY DEVELOPMENT – ELISA

Mouse anti-chicken IL-10 mAb production

- Mouse anti-chicken mAbs were produced by immunising mice with IL-10 recombinant protein[2]:

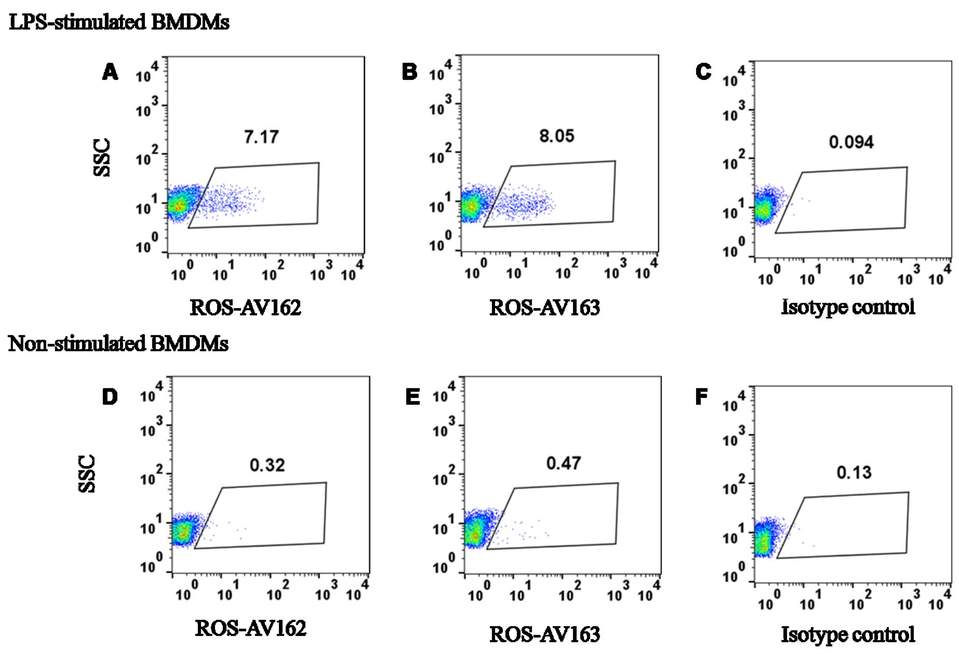

Figure 3: Detection of intracellular chIL-10 in LPS-stimulated chBMDMs. After 7 days culture chBMDMs were stimulated with LPS for 2 hours (A-C) or non-stimulated (D-F) before adding Brefeldin A for a further 4 hours. Cells were fixed, permeabilised and stained for two clones of chIL-10 mAb by flow cytometry, ROS-AV162 (A & D) and ROS-AV163 (B & E). Percentage positive cells are shown. Cells debris was gated out. SSC: side scatter.

ELISA development

- A capture ELISA was developed using the two mAbs:

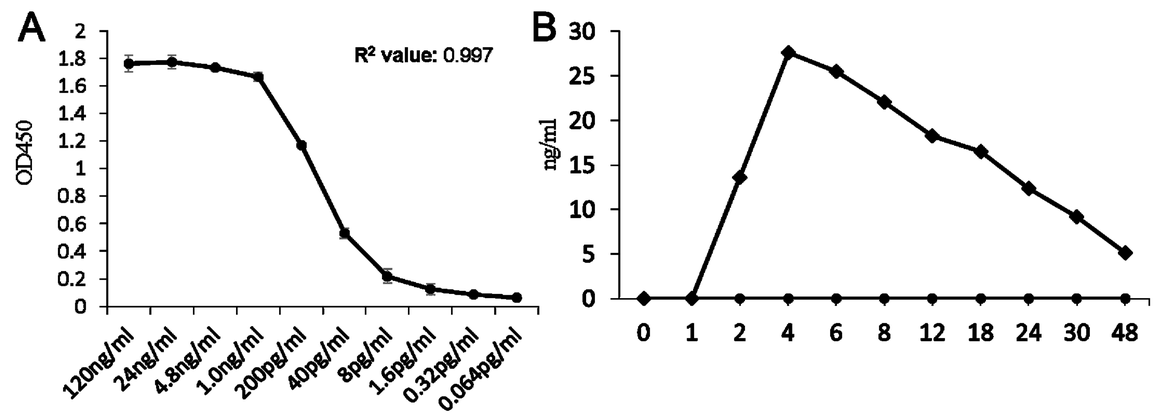

Figure 4. Development of chicken IL-10 capture ELISA. ROS-AV164 as the capture antibody at 4 µg/ml and biotinylated ROS-AV163 at 1 µg/ml as detecting antibody. Recombinant chIL-10-Fc was used as standard (A). Native chIL-10 in LPS-stimulated BMDMs culture in a time course was detected by the described capture ELISA (B). The results were representative of two repeated experiments.

PROJECT SUBMISSION:

- The Immunological Toolbox welcomes enquires and project proposals for the development of new reagents and assays.
- Each project can be individually tailored according to requirements. Additional advice on protocols and procedures is also available through discussion with facility staff and tailored quotes for specific reagent development will be provided according to our agreed costing structure.
- Projects will be prioritised by a steering committee taking into account the nature of the tool(s) requested, their utility, community requirements and accessibility.

CONTACT & FURTHER INFORMATION:

https://www.ed.ac.uk/roslin/facilities-resources/immunological-toolbox

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REFERENCES:

1. WADDELL, L. A., LEFEVRE, L, BUSH, S. J, et al. 2018. ADGRE1 (EMR1, F4/80) Is a Rapidly-Evolving Gene Expressed in Mammalian Monocyte-Macrophages. Frontiers in Immunology, 9.
2. WU, Z et al., 2016. Analysis of the function of IL-10 in chickens using specific neutralising antibodies and a sensitive capture ELISA. Dev Comp Immunol, 63, 206-1