REF: 07/SG/JS

Project Title: Isolation and characterisation of virus-neutralising monoclonal antibodies from hyperimmune pigs

Supervisors: Dr Simon Graham and Dr Julian Seago **Research group:** PRRS Immunology and Molecular Virology

Project Summary:

Broadly neutralising antibodies are the subject of intense recent research in the context of a number of highly variable viruses, such as influenza and HIV. Central to these efforts are methods to generate and analyse the specificity of naturally occurring monoclonal antibodies (mAbs), which may be used as immunotherapeutic agents or as tools to define novel targets for vaccine development. The porcine reproductive and respiratory syndrome virus (PRRSV) is responsible for the most important infectious disease affecting the global pig industry. The rapid evolution of PRRSV poses a major challenge to effective disease control since available vaccines show variable efficacy against divergent strains. Knowledge of the antigenic targets of virus-neutralising antibodies that confer protection against heterologous PRRSV strains would be a catalyst for the development of next-generation vaccines. Key to discovering these epitopes is the isolation of mAbs from PRRSV-immune pigs. To address this important unmet need, an innovative approach is being pursued which involves using a retrovirus expressing key gene regulators to genetically programme memory B cells isolated from immune animals. Programmed B cells are converted into proliferating, antibody-secreting cells which retain surface antibody expression; making them amenable to enrichment, cloning and direct analysis of antibodies in cell culture supernatants.

Details:

The aim of this project is to use this cutting edge technology to identify and characterise naturally occurring porcine mAbs specific to PRRSV. Mononuclear cells have been collected and cryopreserved from the blood and mucosal lymphoid tissues from a cohort of pigs' hyper-immunised by sequential challenge infections with heterologous PRRSV strains, which produced high titres of neutralising neutralizing antibodies. Memory B cells will be isolated and genetically programmed, following enrichment for PRRSV-specific cells by antigen baiting, cells will be cloned and screened for mAbs which neutralise PRRSV infectivity. PRRSV-neutralising mAbs will then be subjected to in-depth analyses. The ability of recombinant mAbs to cross-neutralise porcine alveolar macrophage infectivity by the panel of PRRSV strains will be quantified to identify those with the broadest and most potent activity. To assess the antigen-specificity of broadly-neutralising mAbs we will conduct co-immunoprecipitation experiments with lysates from infected cells and complement with confocal microscopy to visualise mAb binding within virus infected cells. The proposed research will provide an essential first step towards the identification of the viral molecular targets that can then be used to design candidate broadly-protective immunogens.

The successful applicant will be embedded in the PRRS Immunology Group and will receive support and guidance from the group leader Dr Simon Graham. Additional support will be provided by other postdocs and students in the group as well as Dr Julian Seago and the Molecular Virology, whom we share lab space with and hold joint lab meetings.

References for Suggested Reading:

- www.pirbright.ac.uk/viruses/prrsv
- www.pirbright.ac.uk/our-science/livestock-viral-diseases/porcine-reproductive-and-respiratory-syndrome-prrs-immunology
- www.pirbright.ac.uk/our-science/molecular-virology
- B. Charleston, S.P. Graham. 2018. Recent advances in veterinary applications of structural vaccinology. Curr Opin Virol 29:33–38. DOI:10.1016/j.coviro.2018.02.006
- M.C. Rahe, M.P. Murtaugh. 2017. Mechanisms of Adaptive Immunity to Porcine Reproductive and Respiratory Syndrome Virus. Viruses 9: E148. DOI: 10.3390/v9060148.