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Title: The role of African swine fever virus membrane proteins in virus replication and immunomodulation
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Project Abstract

African swine fever virus is a large cytoplasmic DNA virus which encodes many proteins involved in evading the host's defence systems. This project will focus on one class of these proteins which are transmembrane proteins and are therefore predicted to be present on the surface of virus-infected cells. Interaction of the extracellular domain of these proteins with ligands on the surface or secreted from cells provides a mechanism for virus immunomodulation. The project will investigate the function of three ASFV membrane proteins by studying their localisation in cells, identifying ligands that they interact with and the functional consequences of these interactions. The information will be useful for the design of rationally attenuated African swine fever virus vaccines.

Full description of project

African swine fever virus causes an acute haemorrhagic fever and high mortality in pigs but causes persistent infections with no apparent disease signs in its natural hosts, warthogs, bushpigs and soft ticks of the *Ornithodoros* species. This demonstrates that the virus has effective mechanisms to evade the hosts defences and avoid elimination. The virus is a large, cytoplasmic DNA virus and encodes between 160 and 175 genes including many which are not essential for virus replication but have an important role in virus survival and transmission. These include genes which help the virus to evade the host's immune system. Although a number of ASFV immune evasion genes have been identified many more remain to be discovered. This project will focus on a class of ASFV genes which are transmembrane proteins and are either expressed in the membranes of infected cells or on virus particles or both of these. These have the potential to interact with other cells of the immune system or extracellular molecules as well as to transmit signals within infected cells. ASFV replicates primarily in macrophages and modulation of macrophage function is key to the virus induced pathology and to its mechanisms of immune evasion. The project will focus on predicted transmembrane proteins including a virus-encoded C-type lectin protein called EP153R. Proteins containing C-type lectin domains are known to be important regulators of the immune system and are expressed on lymphocytes, dendritic cells, macrophages and neutrophils. Adhesion proteins such as selectins have a critical role in regulating cell migration and attachment and have other functions such as in lymphocyte activation and phagocytosis. ASFV has sophisticated strategies to compromise the host immune system, one possibility is that EP153R may bind to receptors and interfere with

killing by CTL or NK cells or influence cell activation and migration. It is already known that the EP153R protein is not essential for ASFV regulation and virus deletion mutants are available. However the function of the EP153R protein has been little studied. There are several hypotheses to explain how EP153R protein may function and these will be unravelled using the approaches described below. Other virus-encoded membrane proteins of interest include two which are related to the p22 virus structural protein. These are encoded by virulent isolates but not the tissue-culture adapted isolate, suggesting they have a role in virulence or in virus replication in macrophages.

The aims will be to characterise the function of these membrane proteins by; 1) determining the localisation in infected cells, 2) identifying cell types and ligands that the extracellular domains bind to, 3) identifying intracellular pathways and targets that the cytoplasmic tails bind to and 4) determining the effect of these proteins on infected macrophage and bystander immune cell functions. The following approaches will be used. 1) The virus genes will be modified to include different epitope tags at the C-terminus and the N-terminus of the gene. This approach will enable both the N and C-terminal domains of the proteins to be localised by confocal microscopy and by cell fractionation and Western blotting using antibodies against each epitope tag. This will identify if the proteins are expressed on the plasma membrane of infected cells or internal membranes and if there is a secreted form as well as identifying if the protein is incorporated into virus particles. We have already used this approach to study another transmembrane protein, CD2v. 2) To identify cells that the extracellular domain of the proteins bind to secreted forms of the proteins tagged with a motif which can be biotinylated will be constructed the secreted protein can be purified and attached to fluorescent beads. Binding of this protein to different immune cell populations will be measured by FACS using antibodies specific for different cell types. 3) The function of the cytoplasmic domains will be investigated by identifying intracellular proteins to which this domain binds and its effect on signalling pathway activation. This will be investigated using cell lines expressing the individual proteins or infected with wild type or deletion mutant ASFV. 4) The effect of these proteins on the function of infected macrophages will be investigated by infecting pig bone marrow cells with wild type ASFV and deletion mutants. Already described effects of ASFV infection are activation of NK cells, stimulation of B cell proliferation, induction of apoptosis and inhibition of lymphocyte proliferation. Other functions predicted from the binding partners identified will be studied.

Research activity of the Group

The main activities of the African swine fever virus group are to understand the mechanisms by which the virus manipulates the host's defence system by studying the function of virus encoded proteins involved in these activities. This information is being applied to the design and construction of of rationally attenuated virus vaccines.

References to project for further reading

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