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Title: Phenotypic and functional characterisation of cattle natural killer (NK) cell clones
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Project abstract

Natural killer (NK) cells play an important role in innate resistance to many cattle pathogens, including *Mycobacterium bovis*, the causative agent of tuberculosis. They express an array of membrane-bound receptors (encoded by distinct gene families) that largely control the NK response to infection. Many recognise subsets of ubiquitously expressed MHC class I genes, and NK cell function is to a large extent controlled by the balance of expressed inhibitory and activating receptors with shared ligands. MHC class I genetics and expression are complex in cattle, and we have recently demonstrated equivalent complexity in some NK receptor (NKr) gene families. Different clones of NK cells within an individual may each express a distinct complement of receptors. Coupled with extensive haplotype variation in both MHC class I and NKr genes, this suggests a complex interplay in cattle involving NK cells, receptors, their ligands and potentially also pathogens that may subvert MHC expression.

The aim of this project is to gain insight into this interplay, leading to strategies (selective breeding / improved vaccine design) to increase disease resistance in commercial cattle herds. Data generated will also shed light on evolution of the mammalian immune system. The project will involve generation and maintenance of phenotypically distinct sets of NK clones from animals expressing characterised MHC class I haplotypes. NK clones will be characterised using flow cytometry with mAbs recognising NKr, and RT-PCR from cDNA using gene-specific primers. Functional differences between clones will be established by measuring cytotoxicity and cytokine production. The response of NK clones to cells expressing different MHC class I genes will be measured. Changes in expression of NKr genes will be assessed in a small number of MHC-defined animals in response to infection or vaccination. Reagents and data for the work are being generated during the course of on-going projects within the PI's group.

Full description of project

Natural killer (NK) cells constitute key components in the early response to intracellular infections, and influence subsequent adaptive or specific responses to pathogen. They have been implicated in the immune response to intracellular cattle pathogens, such as *Babesia bovis*, *Neospora caninum* and *Mycobacterium bovis*. Understanding the role of NK cells in protective immunity to *M. bovis* for example could provide insights important for targeted vaccination strategies to control this disease which is rapidly increasing in UK cattle herds.

NK cells express a diverse array of membrane-bound receptors encoded by a number of distinct gene families; the killer cell Ig-like receptors (KIR) are encoded by the largest and most complex of these gene families in primates. Cattle have recently been shown to demonstrate equivalent complexity in this gene family; this project will therefore focus primarily on the *KIR* genes. *KIR* genes are polymorphic, encode both activating and inhibitory receptors, and are found in a wide range of combinations in different haplotypes. In primates, most inhibitory KIRs and at least some activating KIRs recognise subsets of MHC class I alleles; the ligands for cattle KIRs have not yet been

identified (work in progress in principal supervisor's group). NK cell function is thus controlled to a large extent by the balance of expressed inhibitory and activating receptors, which in humans has been shown to vary not only between individuals but between clones of NK cells within an individual. Recent work by a current PhD student in the principal supervisor's group has shown that this is also the case in cattle.

Studies in human suggest that the combined repertoire of *KIR* and *MHC* genes forms an important component of the NK-mediated immune response to infectious pathogens; there is clear evidence linking certain combinations of *KIR/MHC* with disease outcome, for example in hepatitis C and HIV infection. Different NK clones within an individual will survey different MHC molecules expressed on infected cells, by expressing a limited subset of the KIR encoded in its genome, and thus will be functionally diverse. This project will address the hypothesis that *KIR/MHC* genotype in cattle influences NK cell functional capability, affecting resistance and susceptibility to the pathogenesis of many diseases. This will be approached by studying NK clones from healthy, infected or vaccinated individuals with distinct *MHC/KIR* genotypes.

The project will utilise the genetically-defined cattle herd at Compton. These animals have defined MHC genotypes and we are currently optimising the tools for *KIR* genotyping. Cattle NK clones have been generated for the first time recently by a group at Edinburgh University, by co-culturing peripheral blood mononuclear cells from naive cattle with autologous *Theileria annulata*-infected lymphocytes (Ivan Morrison, personal communication). We have analysed transcription of individual *KIR* genes in a set of 9 of these NK clones from one individual and demonstrated significant variation. We have generated a monoclonal antibody (mAb) to confirm that surface expression of KIRs also varies between clones.

An initial aim of the project will be to generate and maintain sets of NK cell clones and lines from a small number (2 or 3) of animals with defined *MHC/KIR* genotypes. The clones will be phenotyped using a combination of gene-specific primers and mAbs, and a number of distinct clones will be selected from each animal for further analysis. Functional differences between these clones will be assessed using cytotoxicity assays and by measuring production of cytokines. Although the methods to be used are to some extent established, they remain challenging, and the likely outcome (levels of variation in phenotype and function) is unknown. The University co-supervisor has many years of experience of cloning cattle T cells and of using *T. annulata* lines, and his assistance will be sought if problems arise in this area.

The next stage will be to examine the response of the NK cells to target cells expressing different MHC class I molecules; assays may measure cytotoxicity or cytokine production, or another parameter yet to be defined. It is anticipated that by this stage we will have identified the MHC ligands for some of the KIRs. If this is not the case, we will utilise a large panel of target cells expressing a range of possible ligands. Mouse P815 cells (targets for NK killing) transfected with single MHC class I genes will be used, in addition to cattle cells expressing different combinations of ligands. Data generated in these studies should reveal useful information about the affinity of different KIRs (products of distinct genes or alleles) for different MHC ligands; in human this has been shown to relate very strongly to disease outcome. Other projects within the principal supervisor's group address overlapping questions, thus the precise nature of this part of the project may change to reflect new data. The final part of the project will examine changes in KIR expression in NK cells in animals following infection or vaccination for example using BCG, bovine herpesvirus or FMDV. This will be carried out in collaboration with the co-supervisor Jayne Hope, using animals that are part of other funded studies. A wide range of techniques, encompassing molecular biology, cell biology and functional assays will be applied by the student in this study. Information about the role of *MHC/KIR* combinations in cattle immune responses will be useful for cattle breeding programmes designed to improve animal health, in addition to increasing our understanding of the evolution of the mammalian immune system.

Research Activity of the Group

The work of the bovine molecular immunology group focuses on molecular genetic analysis of MHC and the functionally related NK receptor genes, primarily in cattle. The group has developed and is using techniques to define genetic polymorphisms, and to analyse T cell responses. It is currently additionally focused on defining interactions between NK receptors and

MHC molecules. The broad focus of the group is on evolution of genes involved in both innate and acquired immune responses, and the use of this information to develop improved disease control strategies. There is expertise within the supervisors' groups in a wide range of techniques, spanning molecular biology, cell biology and functional assays. The University co-supervisor has expertise in different but related areas, specifically MHC analysis, investigation of T cell responses and vaccine development in cattle and sheep.

References relating to project for further reading

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