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Title:	Determine the genetic mechanism responsible for generating diversity in the cattle NK cell receptor repertoire
IAH Supervisors:	Dr John Hammond, Dr Shirley Ellis & Michael Watson
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University:	Imperial College, London
University Supervisor:	Dr Salim Khakoo

Project Abstract

Pathogens are constantly evolving to evade and subvert the immune response creating pressure on the immune system to counter-evolve to remain effective. One response has been to duplicate and diversify immune genes. Insight into the resulting immune complexity is crucial to understanding disease resistance and therefore our ability to manipulate immune responses. The killer immunoglobulin-like receptors (KIR) are an extremely diverse natural killer (NK) cell receptor family in primates. Primate MHC class I is also highly variable, and as the ligand for KIR has helped drive KIR diversification. The interaction between KIR and MHC class I controls NK cell function, a critical component of the innate immune system. In humans, particular combinations of KIR and MHC class I significantly increase survival from viruses such as HIV and hepatitis C. MHC class I is more variable in cattle than in primates. Cattle also have a unique and diverse KIR receptor system. It is therefore likely that cattle MHC class I has driven this specific KIR expansion and that interaction between them is crucial for disease resistance.

Our hypothesis is that during cattle evolution, KIR has evolved with their diverse ligands to remain functional. This project will determine if cattle KIR are as variable as the MHC class I and if this mirrors primate KIR/MHC coevolution by ascertaining the extent of KIR diversity and how variation is generated through inheritance. This will be achieved by assembling and characterising the first complete cattle KIR haplotype and by determining KIR gene content in several individual animals. This will involve using a range of molecular biological methods leading to advanced bioinformatic analysis. Depending on the results and the student's interest a KIR genotyping system could be developed or individual KIR function interrogated at the cellular level. This fundamental knowledge will lead directly to investigating the role of KIR in disease resistance.

Full description of project

Natural Killer (NK) cells are blood lymphocytes that function in the innate immune response to infection, providing a particularly important defence against viruses and other intracellular pathogens. This response is crucial for survival as humans lacking functional NK cells succumb to overwhelming viral infection, despite producing an adaptive immune response. The cattle NK cell response is also crucial for defence against pathogens, such as bovine TB. NK cells are controlled by a wide variety of different types of receptors, including several encoded by diverse and rapidly evolving gene families. This diversity is often species-specific and variable within a population, reflecting the enormous impact of pathogen selection and the importance of maintaining a varied immune response. One such gene family is KIR, which has independently diversified in cattle and primates. It was recently determined that cattle KIR are not only polygenic but also polymorphic and of a different ancestral lineage to the diverse primate KIR. The known ligands for most NK cell receptors, including KIR, are MHC class I. The cattle MHC class I region is the most diverse yet characterised and a current project in the co-supervisor's group has shown high MHC diversity in British Friesians. During cattle evolution, MHC class I receptors have had to evolve with these diverse ligands to remain functional. Therefore, cattle KIR is an NK cell antigen recognition system that mirrors the crucial primate system but has evolved entirely independently.

The first aim is to assemble and characterise the first complete cattle KIR haplotype (~ 150 kb) using a British Friesian BAC library available in the Ellis laboratory. A feature of KIR diversification has been episodes of gene duplication followed by functional diversification. This results in multiple areas of high sequence identity and has prevented the assembly of the KIR region in the cattle genome. To overcome this, the BAC library will be screened using cattle KIR DNA probes. DNA from positive clones will be restriction digested to identify those representing a single haplotype. End sequencing will confirm their identity before sequencing using 454 technology. The haplotype will be assembled, incorporating finishing sequencing if necessary, in collaboration with the bioinformatics group. In the unlikely event that this library does not contain the KIR region, KIR-containing clones are commercially available from a CHORI BAC library.

The second aim is to characterise the KIR genes from up to 45 premium British Friesian bulls. A PCR strategy has been developed by the principal supervisor that amplifies all known cattle KIR. This will be applied to gDNA and cDNA to determine KIR receptor content by sequencing. Confirmation of expressed cattle KIR number will be obtained with Northern blotting. The MHC class I genotype of these animals has previously been determined as part of another study. This complimentary dataset will provide an important comparison to KIR diversity in each individual.

These complimentary aims will enable comprehensive phylogenetic and bioinformatic study of cattle KIR. Analysis will include phylogenetic reconstruction, recombination analysis, divergence time estimation and ancestral sequence reconstruction. This can then be compared directly with the known mechanisms of primate KIR evolution to highlight common and variable components. Novel gene sequences isolated in aim two can be extended to enable accurate mapping onto the cattle haplotype. This will pinpoint recombination areas within the KIR gene cluster and decipher how KIR haplotypes vary. This is a particularly important mechanism of maintaining KIR diversity in primates. Determining the extent of KIR gene polymorphism in British Friesians will enable a direct comparison with MHC class I diversity to determine if selective breeding has reduced KIR diversity. These data will also facilitate selection analysis to determine the residues under the greatest positive selection and therefore those likely to influence ligand binding. An ongoing project is currently determining which MHC class I molecules bind individual KIR. This will allow polymorphisms predicted to influence ligand binding to be tested in vitro using cytotoxic assays. Functional data could then be applied to the British Friesian KIR and MHC class I genotype to identify the extent of receptor and ligand match and mismatch.

This project will significantly advance our knowledge of the complex cattle NK cell receptor recognition system. It has the potential to confirm KIR as key to the immune response and indicate the importance of individual receptors. Such detailed knowledge will enable the future development of accurate genotyping methods to characterise KIR content and diversity in entire herds and across breeds. The role of KIR genotype in disease resistance can then be explored with confidence that the range of genetic diversity is incorporated.

Research activity of Research Group

The principal supervisor is experienced in molecular biological techniques and bioinformatic/phylogenetic analysis. Current projects are exploring the evolution of ruminant KIR, identifying the MHC class I ligands of cattle KIR and profiling the expression of cattle KIR. The principal supervisor is currently working in conjunction with the bovine molecular immunology group, whose broad focus is to study the evolution of genes involved in both the innate and acquired immune response, and the use of this information to develop improved disease control strategies. This currently includes MHC class I and several NK cell receptors. Within this group there is also considerable expertise in a wide range of molecular and cellular techniques.

References to project for further reading

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