

Innate Resistance as an Evolving Paradigm Against Select Agents of Livestock Species

| | | | | |
|--|--|--|-------------------------|--|
| DHS Priority Areas Addressed | <input checked="" type="checkbox"/> Prevention <input type="checkbox"/> Detection <input type="checkbox"/> Response <input checked="" type="checkbox"/> Recovery <input type="checkbox"/> Education/Risk Communication | | | |
| Proposal Section Addressed | Section 5.1.1 | | | |
| Investigators | TAMU: James Womack and Garry Adams | | | |
| Objectives | Deliverables | Progress Toward Deliverables | Percent Complete | |
| Map bovine TLR and NOD genes | Map the TLR and NOD gene families (consisting of 10 TLR and 2 NOD genes) to specific location on bovine chromosome | We have completed the radiation hybrid mapping of the TLR and NOD genes in cattle. Our map will be used to annotate the rapidly emerging bovine genome sequence. | 100 | |
| Sequence bovine TLR and NOD gene | Sequence all known TLR and NOD genes in cattle | All bovine TLR genes have been sequenced. We have submitted these sequences to Genbank where they are now available to the public. We have done extensive resequencing to resolve sequence divergence and allelic structure across cattle breeds. | 100 | |
| Conduct SNP analysis of bovine TLR and NOD genes | SNP analysis for all genes in 9 cattle breeds | We have done SNP analysis with our breed panel and the Hereford sequence in Genbank. We have sequenced approximately 35 kb of DNA from each animal in our nine breed panel. We have focused the 9 breed screen to coding regions where we have now found a total of 254 SNPs, 47 of which are predicted to be amino acid altering. | 97 | |
| Identify candidate genes for resistance to RVF | Identification of gene(s) for RVF resistance in rats | We have completed a low resolution genome scan of the congenic resistant strain and identified two candidate regions of the rat genome for the resistance gene. We have successfully rederived a colony of congenic resistant rats and begun arrangements for RVF virus testing with Dr. C.J. Peters. Meanwhile we have expanded the markers employed for a higher resolution scan of the two congenic regions of interest. We have clarified the distribution of alleles in substrains of LEW which may explain discrepancies in reported strain distribution of resistance in inbred rats. | 55 | |
| | Identification of putative gene(s) for RVF resistance in livestock species | Identifying the congenic regions has provided a plethora of potential genes from the rat/bovine comparative map, although none are obvious candidates. We have begun narrowing the congenic regions and consequently, the number of viable candidate genes for RVF resistance in livestock species. | 10 | |

Highlight for Research Briefs

Our success with the discovery of variation in bovine TLR 3, 7, 8, and 9 has been published (Cargill, E.J. and Womack, J.E. 2007. Genomics 89: 245-255) and a paper describing variation in bovine TLRs 1, 5, and 10 is forthcoming (Seabury, C.M., Cargill, E.J., and Womack, J.E. 2007. Genomics, in press). These data were presented by Womack in invited lectures at the 20th International Mammalian Genome Conference (Charleston, S.C.) in 2006 and at the 8th International Veterinary Immunology Symposium (Ouro Preto, Brazil) in 2007.

Our principal highlight in RVF resistance is the successful rederivation of a congenic strain of rats that has existed only as frozen embryos for the past 20 years. The animals are reported to carry a gene for RVF resistance by backcrossing from a resistant to a susceptible strain. We have resolved several issues of substrain divergence which may explain conflicting published results on resistance to the RVF virus in inbred rat strains (Callicott, R.J., Ballard, S.T., and Womack,

J.E. 2007. J Am Assoc Lab Anim Sci 46: 25-29) Having the gene in a congenic strain will facilitate its identification through genetic mapping and molecular genetic technology.

Interpretive Summary

Animal populations generally carry a wealth of genetic diversity expressed in the form of differential response to specific pathogens. These genetic differences in innate resistance to disease are important to us for at least two reasons. The identification of specific genes underlying resistance to a specific pathogen will allow rapid expansion of numbers of resistant animals, either by natural breeding enhanced by marker assisted selection, or by emerging reproductive biotechnologies. A second and perhaps even more important reason to find genes underlying pathogen resistance is to dissect and understand the vast network of host-pathogen interaction and cell signaling pathways involved in an animal's disease defense mechanisms. Better understanding of these mechanisms will provide a foundation for improved vaccines and therapies. We have completed the deliverables associated with our first three objectives, namely we have mapped and sequenced the bovine TLR and NOD gene families and identified naturally occurring variation in all genes. We now have candidate SNPs for association studies in cattle exposed to FMD or RVF.

In another study, we are building on preliminary work of Dr. C.J. Peters and colleagues who identified innate resistance to RVF virus in a rat model. Using DNA from a congenic strain in which the resistance gene was backcrossed onto a sensitive strain, we have identified two segments of the rat genome which carry markers of the resistant strain. Thus, the gene probably lies in one of these two chromosomal regions, RNO3.43 (the distal end of chromosome 3) or RNO9.13 (near the centromere of chromosome 9). Neither region contains obvious candidate genes, although work remains to be done to annotate these regions. Identification of this gene will provide a model for resistance to the RFV virus in other mammals.

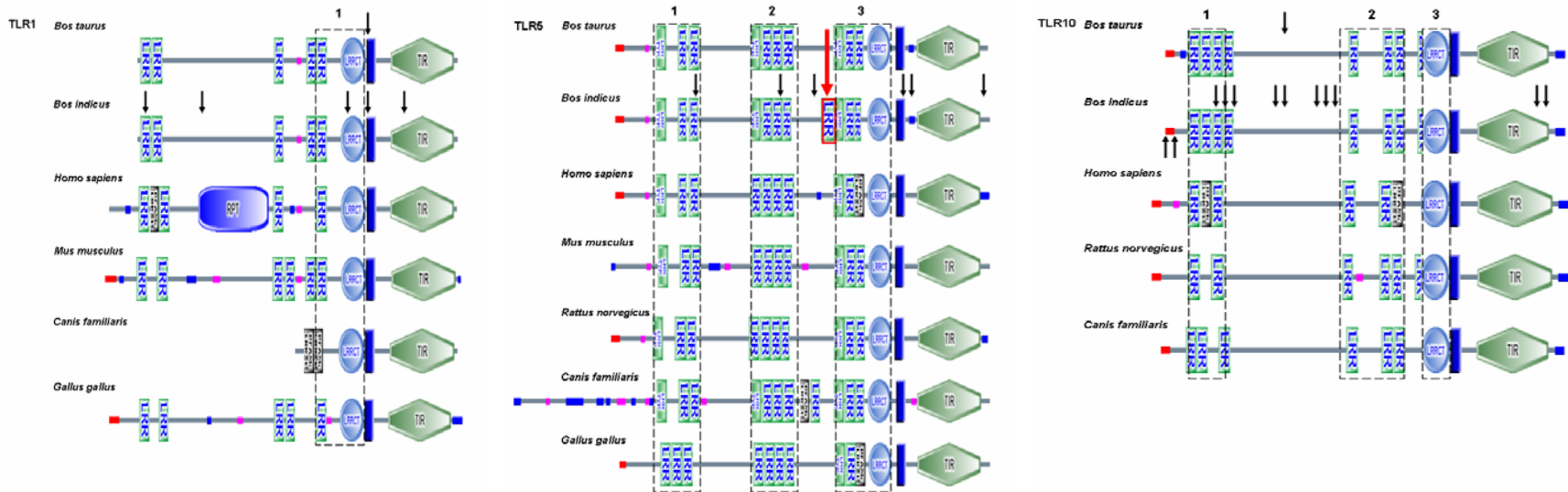
Results and Interpretations

The scope of this project is to produce specific biological research outcomes with intermediate and longer term applications for prevention and recovery from bioterrorist or accidental events related to FMD, RVF, and BRU. Highly sophisticated analyses of host genomes are proposed to identify the genetic basis for innate resistance to select agents. The identification of specific allelic variation that predisposes individuals to differential response to pathogens will 1) facilitate our understanding of host-pathogen interactions, 2) provide DNA markers for selecting naturally resistant animals and 3) provide genes for engineering of livestock genetically resistant to targeted pathogens.

Toll-like receptors are cell-surface signaling molecules that activate innate immune responses in mammals. The TLR family in mammals presently consists of ten known members (only nine to date in mice) and more are probably forthcoming. While the signaling domains of the toll-like receptors known in mammals are highly conserved, the leucine-rich repeat (LRR) ligand-recognition domains are more diverse to accommodate recognition of different pathogen-associated molecular patterns (PAMPs). TLRs have unequivocally been shown to participate in the recognition of microbial components and the activation of innate immunity, leading to the development of antigen-specific immune responses. A second family of proteins that have been implicated in the recognition of pathogen components share a nucleotide oligomerization domain and have thus been termed the NODs.

We have mapped the TLR and NOD genes in cattle to their respective chromosomes. We have placed TLR1, 6, and 10 on chr 6; TLR2 on chr 17; TLR 3 on chr 27; TLR 4 on chr 8, TLR 5 on chr 16, TLR 9 on chr 22, and TLR 7 and 8 on the X-chromosome. NOD1 is on chr 4 and NOD2 is on chr 18. Partial sequencing and SNP analysis has revealed 254 SNPs in TLR genes, 47 of which alter amino acid structure of the respective receptor molecule. This is in addition to the comprehensive SNP discoveries we previously published for TLR2 and 4 and NOD2. Thus, we have defined the TLR and NOD gene families at the genome level and have demonstrated sufficient polymorphism in individual genes to provide a wealth of genomic diversity in breeding populations of cattle. These data will permit retrospective studies of experimentally infected herds to determine the relative contribution of individual TLR and NOD genes to differential responses. As illustrated in the figure below drawn from unpublished data (Genomics, in press), we have found several amino acid altering SNPs in TLRs 1, 5, and 10, particularly in comparisons between *Bos taurus* and *Bos indicus* breeds (black arrows). The large (red) arrow in TLR5 identifies to location of a SNP that is predicted to alter the structure of the LRR domains, making it a prime candidate for differential response to TLR5-associated PAMPs. Domain structure of

homologous TLRs in other species are predicted from available sequence data for comparison with the bovine receptors:



In collaboration with Dr. C.J. Peters, U.T.M.B., Galveston, we have begun work to identify and positionally clone the gene responsible for resistance to the Rift Valley Fever Virus in laboratory rats. A congenic rat strain developed by Dr. Peters and no longer available as a breeding colony is being rederived from cryopreserved embryos at the NIH. New tools developed from the rat genome sequencing project have been employed to identify a congenic segment of the genome containing the resistance gene. We have designed primers for PCR analysis for a genome scan to identify the congenic segment. The first genome scan revealed two congenic segments, one in band 43 of rat chromosome 3 and one in band 13 of chromosome 9. Additional primers are being developed to further characterize these regions. No obvious candidate genes present themselves in these chromosomal segments. Rederivation of congenic embryos at the University of Missouri was achieved in the summer of 2006 and breeding and backcrossing are underway with plans to ship rats to Galveston in the fall of 2007 for RVF virus challenge.

Technology Transition

We are in the process of writing a proposal for funding the construction of an oligo microarray for SNP detection in candidate genes for disease resistance in cattle. If successful, we will use TLR, NOD, and other data to generate a commercially available chip for rapid detection of candidate genes in populations of animals responding differentially to experimental or natural challenge to infectious agents.

Status of Funding

We reported some carryover funds which we propose to use to complete Objective 4. We will also write new proposals for functional analysis of TLR and NOD SNPs and to continue the quest for the RVF resistance gene.