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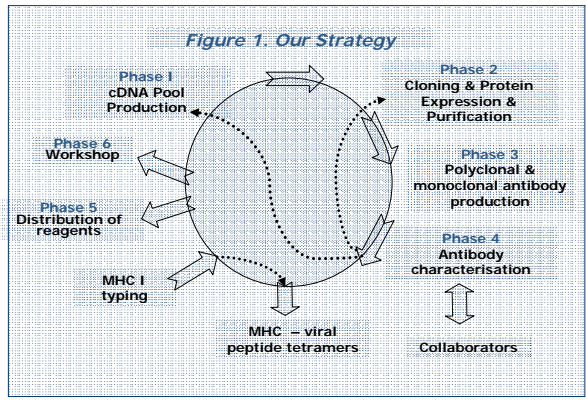
## THE IMMUNOLOGICAL TOOLBOX: EQUINE REAGENTS

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### INTRODUCTION

Progress in understanding and utilising the immune response of livestock animals to pathogens and vaccines has long been limited by lack of appropriate reagents. There is a particular gap in suitable reagents to identify, isolate and manipulate immune molecules such as cytokines and immune cell populations, such as antigen specific T cells and antigen presenting cells. This project addresses the problem by a co-ordinated effort to develop reagents to study protective and pathological responses in horses, cows, sheep, pigs and chickens. This "Immunological Toolbox" is organised by three institutes, the Animal Health Trust, Moredun Research Institute and Institute for Animal Health, Compton. This poster presents the current status of progress in the generation of equine specific reagents for application to viral diseases.



### AIMS

1. to generate antibodies against equine molecules of key immunological interest - see Figure 1 & Table 1.
2. to develop a rapid technique to identify MHC class I B2 positive horses - see Figure 2 and Figure 3.
3. to construct tetramers involving the equine MHC class I B2 gene and CTL target peptide in Equine herpesvirus-1 gene 64

### RESULTS TO DATE

1. Cloning of DNA sequences encoding target antigens is well underway and reagents are beginning to emerge - see Table 1.
2. A PCR which specifically amplifies the MHC class I B2 gene product has been characterised and applied to identify horses with this genotype for vaccine trials - see Figure 2.
3. A PCR which amplifies a conserved region within classical MHC class I is under development. Sequencing of these products from horses which express homozygous serological haplotypes will be used to characterise MHC variation further - see Figure 3.
4. Fragment cloning of EHV-1 gene 64, to identify regions which encode CTL target peptides is in progress. These peptides will be used to construct MHC class I - peptide tetramers and ultimately to characterise peptide specific immune responses *ex vivo*.

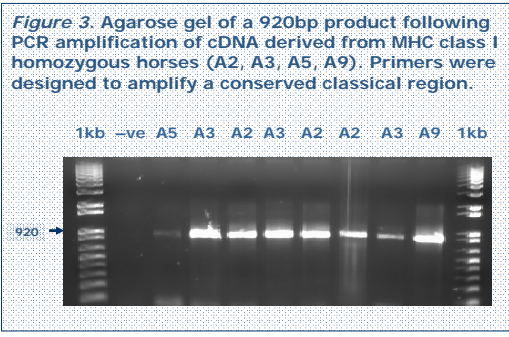
"Twilight": an MHC class I A3 homozygous mare. She donated DNA for the Equine Genome Sequencing Project and Aim 2 of this study.



Photo courtesy of Prof. Doug Antczak, Baker Institute, Cornell University, USA.

Table 1. Antigens selected and current status of cloning, expression & reagent production

Antigen	Prokaryotic expression system*	Eukaryotic expression system*
TNF $\alpha$	Cloned, recombinant protein expressed & polyclonal antiserum available	Cloned & CHO cells transfected with plasmid
IL15	Immunisations underway	Transfections in progress
IL6	Recombinant protein purified, immunisations pending	Transfections in progress
IL17	Recombinant protein purified, immunisations pending	Amplification failed
Granzyme B	Induction in progress	Cloned & sequence verification under way
CD14	Amplified by PCR	Amplified by PCR
Transferrin	Cloned & sequence verification under way	Cloned & sequence verification under way
Lactoferrin	Amplified by PCR	Amplification failed
RANTES	Purifying recombinant protein	Transfections in progress
TLR4	Cloned & sequence verification under way	Cloned & sequence verification under way



**Additional Collaborators:**  
 Cornell University: Dr B Wagner, Prof D Antczak;  
 University of Glasgow: Dr L Nicolson;  
 University of Kentucky: Dr D Horohov

**Related links**  
 The Immunological Toolbox <http://www.immunologicaltoolbox.co.uk>  
 USDA-funded project <http://www.umass.edu.velimm/>  
 Equine Genome Sequencing Project <http://www.genome.gov/20519480>

\* Sequences and frames were verified prior to expression