

The Immunological Toolbox: Ovine Reagents

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Introduction



The SEERAD component of the Immunological Toolbox funds a post at MRI to assist in the development of tools and reagents to study immunology in sheep. This is part of a wider consortium funded collaboratively between BBSRC and SEERAD to develop immunological reagents for cattle, pigs, chickens (IAH Compton; Dr J Kaufman, Dr P Kaiser, Dr J Hope) and horses (AHT Newmarket; Prof D Hannant, Dr J Kydd).



Institute for Animal Health



Aims and objectives

The development of reagents and techniques that facilitate studies on immune activation and immune regulation in sheep, in particular:

1. Expression of recombinant cytokines.
2. Development of quantitative assays to measure cytokine production and immune regulation at the molecular and protein level.
3. Generation and characterisation of a flock of MHC class I-typed sheep.

Progress and results

1. Recombinant cytokine expression

We have expressed biologically-active recombinant cytokines in Chinese hamster ovary (CHO) cells using the Celltech proprietary pEE14/GS expression system©. This system has also been used to express bovine cytokines for our partners at IAH (Table 1). Objectives 1 and 2 are aimed at expressing, defining and producing cytokines, producing monoclonal antibodies and the development of ELISAs and intracellular cytokine staining.

Cytokine/Chemokine	ELISA	Intracellular staining
Ov IL-1β	In development	
Ov IL-2		+
Ov IL-3		
Ov IL-4	+	+
Ov IL-5		
Ov IL-6		
Ov IL-8	In development	
Ov IL-10	+	
Ov IL-12	+	
Ov TNF-α	+	+
Ov GM-CSF	+	+
Ov IFN-γ	+	+
Ov RANTES		
Ov MCP-1		
Ov MIP-1α		
Bov IL-2		
Bov IL-4	+	
Bov IL-10	+	
Bov IFN-α		

Table 1: Transfected CHO cell lines developed and established at MRI expressing ruminant cytokines/chemokines. * Monoclonal antibody pairs produced at IAH against the bovine cytokine that are cross-reactive with ovine. ** Commercially-available monoclonal-antibody based bovine ELISAs additional to those at IAH that also cross-react with sheep. For intracellular staining, in several cases only one monoclonal antibody is available.

2. Quantitative PCR for ovine cytokines

Technology has been developed within the Toolbox to quantify expression of mRNA encoding IFN-γ and IL-10 in antigen-driven and mitogen-driven ovine T cells. Moreover, this has been compared to protein expression, and it can be seen that the kinetics of expression of these cytokines differ over a 96hr period (Figure 1).

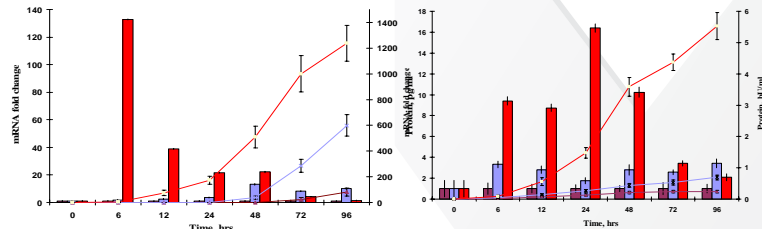
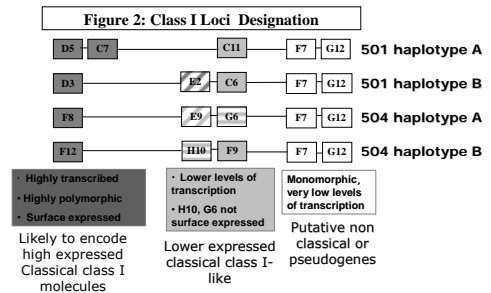


Figure 1. Kinetics of IFNγ (a) and IL-10 (b) mRNA expression (bars, left axis) and protein expression (lines, right axis) following antigen-driven (Ova, purple) or mitogen-driven (ConA, red) activation. Unstimulated control cells are shown in burgundy.

3. Ovine MHC

A diagrammatical representation of the repertoire of class I genes within four ovine MHC haplotypes is shown in Figure 2. MHC homozygous sheep representing each of these haplotypes have been purpose bred as part of the development of the Moredun MHC resource flock. Monoclonal antibody reagents are currently being developed against the products of the highly polymorphic and highly expressed classical class I genes F8 and F12 by immunisation of mice with peripheral blood mononuclear cells from MHC homozygous sheep followed by boosting and screening with transfected cells. This animal resource will permit detailed analyses of CD8 responses in sheep.



Discussion

This collaboration has greatly facilitated knowledge transfer and technology transfer between the partners. The reagents and technologies being developed are underpinning research into disease pathogenesis and contribute to the implementation of disease control strategies in veterinary species.

Future strategies

Cytokine expression and monoclonal antibody production will continue with a focus on the gaps in Table 1. The protein assays will be complemented by further quantitative PCR development. Evaluation of cross-reactive reagents and technology transfer within the consortium will continue. Future priorities will take into account progress on the USDA-funded VIRN initiative, commercially-available reagents and other initiatives such as IUIS/VIC Toolkit, to avoid duplication of effort.

Acknowledgements

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