Generation of novel reagents - the Immunological Toolbox

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Introduction

It is widely accepted that research in veterinary medicine has been hampered due to lack of appropriate reagents, tests and procedures as compared to human medicine, particularly to understand and manipulate the molecules and cells of the immune system. To address this, the BBSRC funded the Immunological Toolbox, as part of the CVDL initiative, to develop reagents for five livestock species (horses, cows, sheep, pigs and chicken). At IAH we have been developing reagents (monoclonal antibodies (mAbs) and recombinant proteins) in the chicken, cow and pig.

Aims

1. To develop reagents (recombinant proteins and mAbs) to cytokines and cell surface markers.
2. Identification of new cell surface markers, particularly on dendritic cells (DC) and different subsets of T cells, and development of reagents thereto.
3. Commercialisation of the reagents.

1. Production of mAbs and recombinant cytokines

Immunisation of mice (C57/BALB/c) i/m (3-5X) with endotoxin-free recombinant plasmid DNA

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Test the sera by ELISA

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Boost i/p with recombinant protein (COS cell supernatant)

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Remove the spleen for fusion with myeloma cells

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Clone positive hybridomas by limiting dilution

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Characterise by dot blot, western blot, bioassay, FACS analysis and capture ELISA

Table 1: Target chicken cytokine molecules for mAb generation

	Signature cytokines	mAbs available	Target mAbs
Innate	IL-1β, IL-6, IFN-α, IFN-β, inflammatory chemokines	IL-1β, IL-6, IFN-α, IFN-β, inflammatory chemokines	IL-1β, IL-6, IFN-α, IFN-β, inflammatory chemokines
Th1	IFN-γ, IL-2, IL-12, IL-18, IL-22	IFN-γ, IL-2, IL-12β, IL-18, IL-22	IFN-γ, IL-2, IL-12β, IL-18, IL-22
Th2	IL-4, IL-5, IL-13, IL-19	IL-4, IL-5, IL-13, IL-19	IL-4, IL-5, IL-13, IL-19
Treg	IL-10, TGF-β4	IL-10, TGF-β4	IL-10, TGF-β4

Table 2: Anti-chicken mAbs generated to date

	Cytokine	mAbs Generated (Isotypes)
Innate	IL-6	3 (IgG2a)
Th1	IL-22	2 (IgM and IgG1)
Th2	IL-4 IL-13 IL-19	6 (2x IgM, 2x IgG2b, IgG1 and IgG2a) 4 (2x IgG2a, IgG1 and IgM) 1 (IgG2b)
Treg	IL-10	1 (IgG1)

Table 3: Chicken cytokines and chemokines. The majority are available as recombinant proteins.

Cytokine family	No. of genes Human	Chicken
Interferons	18	14
Interleukins	39	28
Transforming growth factors	3	3
Tumour necrosis factors	19	10
Colony stimulating factors	3	2
Chemokines		
XCL	2	1
CCL	28	14
CXCL	16	8
CX3CL	1	1
Total	**129**	**81**

2. Identification of new chicken cell surface markers

We are targeting molecules specific to DC (e.g. Flt3), monocyte-derived cells (including DC) (e.g. CD14) and T cell subsets (e.g. CD25). The major T-cell costimulatory molecules and their ligands in chickens have been identified and monoclonal antibodies and fusion proteins, produced for the analysis of their functional roles, are currently being evaluated. A monoclonal antibody recognising chicken CD40 is also available.

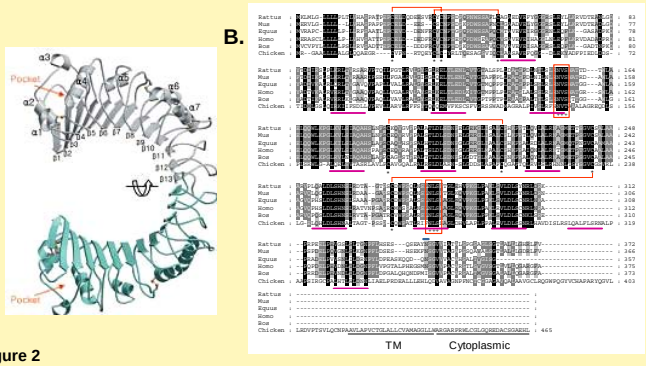

Figure 2
(A) Crystal structure of human CD14. (B) Alignment of chCD14 with mammalian CD14 molecules. ChCD14 is not GPI-anchored unlike mammals, but has a transmembrane domain and a cytoplasmic tail. Red lines joining cysteines show the disulphide bonds, all of which are conserved in the chicken. Red boxes highlight the conserved N-glycosylation sites. Purple lines indicate the LRRs (11 in mammals, 12 in the chicken). The short blue line is the GPI-anchor point in mammalian CD14, not conserved in chicken CD14.

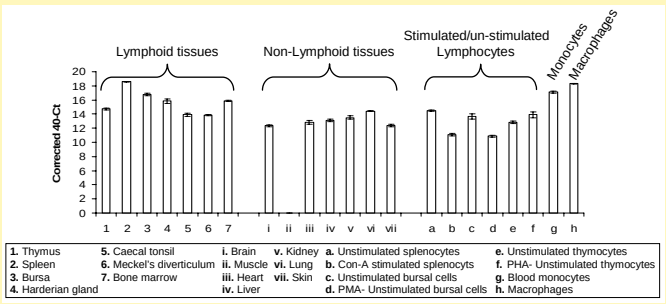

Figure 3
Expression pattern of chCD14 in tissues and cell populations by real-time quantitative RT-PCR.

3. Commercialisation of reagents

Some reagents are already available from Serotec, others will follow.

4. Other species

Expression constructs to make tetramers for porcine class I molecules have been made and distributed to interested parties, along with peptides expected to bind them.
Priorities for cattle are the generation of monoclonal antibodies with specificity for toll like receptors 2 and 4, interleukin-2 and CCR7.

5. Website

A website with an underlying database has been constructed (www.theimmunologicaltoolbox.co.uk); this will provide an invaluable resource for veterinary immunologists.

6. Future work

1. Characterisation of all mAbs for utility in western blot, neutralisation assays, capture ELISA, FACS analysis etc.
2. Clone and characterise chicken cell surface markers (Flt3, CD14, CD34 and CD25) and raise mAbs thereto.
3. Continued commercialisation of reagents through commercial partners (e.g. Serotec).