



U.S. Veterinary Immune Reagent Network



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OBJECTIVES OF VETERINARY IMMUNE REAGENT NETWORK

A major obstacle to advance veterinary immunology and animal disease research is the lack of adequate immunological reagents specific for **poultry, ruminants, swine, equine, and aquaculture species**. Availability of immunological reagents including monoclonal antibodies (mAb) and polyclonal antibodies which can identify the major leukocyte subpopulations (T and B lymphocytes, NK cells, neutrophils, macrophages, dendritic cells) for many animal species including fish will significantly enhance the progress of animal science research. In addition, it is crucial to develop recombinant cytokines and chemokines, as well as antibodies to them and to their receptors, to understand the major components of immune system which are involved in inflammation, innate and adaptive immunity.

These immunological reagents will be used to:

- (1) evaluate changes associated with diseases and vaccination,
- (2) manipulate various lymphocyte subpopulations to evaluate their roles in protective immunity as well as in immunopathology.

Development of these immunological reagents will address the **USDA-CSREES National Research Initiative** goal of enhancing the safety of the Nation's agriculture and food supply by aiding in the development of vaccines. This project represents a broad community plan to begin to systematically address the immunological reagent gap for the U.S. veterinary immunology research community for the following groups: ruminants (concentrating on cattle primarily, but also evaluating sheep, Dr. C. Baldwin), swine (Dr. J. Lunney), **poultry (primarily chickens with some evaluation of reagents for turkeys, Dr. H. Lillehoj)**, horses (Dr. D. Horohov), and aquaculture species (concentrating on channel catfish, Dr. N. Miller and salmonid trout, Dr. J. Hansen). Until recently, only a limited number of immune regulator and effector genes and their encoded polypeptides were identified in avians due to the low level of sequence homologies with their mammalian counterparts. However, with the advent of the chicken genome project, many new genes encoding avian immune-related molecules have been identified.

The goal of this project is to develop 20 reagents per each species group including antibodies that function in ELISA and ELISpot assays, for intracellular staining, for blocking function and signaling, for flow cytometric analysis, as well as for immunocytochemistry using tissue sections.

Products developed in this project will benefit a large group of researchers including veterinary immunologists, pathologists, and microbiologists. This poster will present immunological reagent development efforts for poultry only.

AVAILABLE REAGENTS FOR POULTRY AND ACCESSIBILITY

Accessibility	Protein	Details	Source
2,3	IFN- α	r-protein in <i>E. coli</i> mAb (1,3) polyclonal Ab (1)	¹ Abcam ² BBSRC-IAH ³ AbD Serotec ⁴ USDA-ARS Beltsville* ⁵ Intervet International BV
2,3	IFN- β	r-protein in <i>E. coli</i> polyclonal Ab(2)	¹ BBSRC-IAH ² AbD Serotec ³ Intervet International BV
2,3	IFN- γ	r-protein in <i>E. coli</i> polyclonal Ab(1)	¹ AbD Serotec ² USDA-ARS Beltsville* ³ Intervet International BV

AVAILABLE REAGENTS AND ACCESSIBILITY

Accessibility	Protein	Details	Source
2,3	IL-1b	r-protein in <i>E. coli</i> polyclonal Ab(1,3)	¹ Abcam ² BBSRC-IAH ³ AbD Serotec ⁴ USDA-ARS Beltsville* ⁵ Intervet International BV
2,3	IL-2	r-protein in <i>E. coli</i> mAb(1,2)	¹ AbD Serotec ² USDA-ARS Beltsville
3	IL-3, IL-13, GM-CSF	r-protein in <i>E. coli</i>	BBSRC-IAH
3	IL-4, IL-10	r-protein in <i>E. coli</i>	BBSRC-IAH USDA-ARS Beltsville
2,3	IL-6	r-protein in <i>E. coli</i> polyclonal Ab(1,2) mAb(3,4)	¹ Abcam ² AbD Serotec ³ USDA-ARS Beltsville ⁴ Hiroshima University, Japan
3	IL-8	r-protein in <i>E. coli</i>	BBSRC-IAH (Pete Kaiser) USDA-ARS Beltsville
3	IL-12p35	r-protein in <i>E. coli</i>	Intervet International BV USDA-ARS Beltsville
3	IL-12p40	r-protein in <i>E. coli</i>	Intervet International BV (Netherlands) BBSRC-IAH USDA-ARS Beltsville
3	IL-12p70	r-protein in <i>E. coli</i>	Intervet International BV USDA-ARS Beltsville
3	IL-15	r-protein in <i>E. coli</i> mAb	USDA-ARS Beltsville
3	IL-16	r-protein in <i>E. coli</i>	USDA-ARS Beltsville
3	IL-17	r-protein in <i>E. coli</i> mAb	USDA-ARS Beltsville
3	IL-18	r-protein in <i>E. coli</i>	University of Freiburg (Germany) USDA-ARS Beltsville
2	growth hormone	r-protein in <i>E. coli</i>	ProSpec-tany technoGene
3	lymphotactin	r-protein in <i>E. coli</i>	BBSRC-IAH DNAX Research Institute USDA-ARS Beltsville
3	TGF- β	r-protein in <i>E. coli</i>	BBSRC-IAH
3	TNFSF15 (TL1A)	r-protein in <i>E. coli</i>	Tohoku University, Japan USDA-ARS Beltsville
2	TGF- β 3	r-protein in <i>E. coli</i> polyclonal Ab(1,2)	¹ Abcam ² Developmental Studies Hybridoma Bank

* USDA-ARS in Beltsville

CYTOKINE GENES CLONED AND SEQUENCED IN DR. LILLEHOJ'S LAB (2006-2007)

Gene	Vector	Nucleotide Accession #	Size (ORF)	References
IL-2	pcDNA	AF017645	432 bp	Miyamoto et al (2001)
IL-15	pcDNA3	NM_204571	564 bp	Lillehoj et al (2001)
IL-16	pcDNA3	AJ508678	1824 bp	Min & Lillehoj (2004)
IL-17	pSPORT6	AJ493595	510 bp	Min & Lillehoj (2002)
IFN γ	puc18	AH009942	481 bp	Yun et al (2000)
TNFSF15	pET32a	NM_001024578	720 bp	Park et al (2007)
NK-lysin	pET32a	DQ186291	423bp	Hong et al (2006)
LITAF	pET32a	AY765397	447 bp	Hong et al (2006)
IL-4	pET32a	NM_001007079	411 bp	
IL-10	pET32a	NM_001004414	528 bp	

COMMERCIALY AVAILABLE ANTIBODIES FOR POULTRY

Accessibility	Against	Details	Source
2	CD3, CD4, CD5, CD8a, CD8b, CD28, CD45, TCR $\gamma\delta$, Bu-1, Bu-1a, Bu-1b, TCR $\alpha\beta$ /V β , CD44, TCR $\alpha\beta$ /V β , Ia, IgM, IgG (H&L), IgA, IgY, Ig λ -like light chain, MHC class I, MHC class II, β 2-microglobulin of MHC class I, c-kit, ChT1, monocytes and macrophages	mAb	Southern Biotech
2	Bu-1a, CD28, CD3, CD3 epsilon, CD4, CD41/CD61, CD45, CD5, CD51/61, CD8a, MHC class II, ChT1	mAb	AbD Serotec
2	IgA, IgG, IgG(Fc), IgG(H&L), IgM	polyclonal Ab	AbD Serotec
2	IgG (H&L)	polyclonal Ab	BioSource
2	IgY	polyclonal Ab	Aves Lab. Inc R&D System
2	IgY	mAb	Aviva Systems Biology, Genway
2	IgA, IgG, IgG (H&L), IgM	polyclonal Ab	Bethyl Laboratories
2	CD79a, IgG	polyclonal Ab	Abcam
2	Bu-1a, b, CD146, CD3, CD4, CD5, CD8, ChT1, MHC class I, MHC class II, CD146, CD28, CD44, CD45, CD51/61, CD79a, CD8a, CD1, monocytes and macrophages, TCR $\alpha\beta$, TCR $\gamma\delta$, Vimentin,	mAb	Abcam
2	CD44, MHC class I, MHC class II, Vimentin	mAb	Developmental Studies Hybridoma Bank
2	IgY, IgA, IgM, IgY (H&L), CD3, CD4, CD41/61, Vimentin, TGF β receptor	polyclonal Ab mAb	GeneTex
2	IgG	polyclonal Ab	Invitrogen
2	IgY, IgA, IgG, IgG(H&L), IgM, CD79a, Vimentin	polyclonal Ab mAb	Novus Biologicals
2	IgY, Immunoglobulin	polyclonal Ab mAb	QED Bioscience Inc.

Accessibility Level:

- 1 = Totally available- e.g. ATCC, or distributed without strings.
 - 2 = Commercially available, thus accessible at cost.
 - 3 = available through collaborative agreement only.
- r = recombinant protein

GENES CURRENTLY BEING SEQUENCED AND CLONED IN DR. LILLEHOJ'S LAB (2007-2008)

Gene	Vector cloned	Protein Accession Number (NCBI)	Nucleotide Accession #	Size (ORF)
IL-1 beta	pcDNA1	CAA75239	Y15006	804 bp
IL-2 receptor γ	pcDNA3	NP_989927	NM_204596	636 bp
IL-18	pcDNA3	CAB96214	AJ277865	597 bp
Lymphotactin	pcDNA3	AAB99904	AF006742	294 bp
CCL4 (MIP-1 β)	pBluescript-SK	NP_001025531	NM_001030360	273 bp
CCL20 (MIP-3 α)	pET32a	NP_989769	NM_204438	303 bp
CXCR4	pcDNA3	NP_989948	NM_204617	1077 bp
IL-12p35	pET32a	NP_998753	NM_213588	618 bp
IL-12p40	pET32a	AAP86972	AY262752	948 bp
IL-17D	pET32a		EF570583	351 bp

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