

## INTRODUCTION AND OBJECTIVES

To advance veterinary immunology and animal disease research, a CSREES-funded NRI consortium grant (#2005-01812) was established in 2005 to develop immunological reagents specific for **poultry, ruminants, swine, equine and aquaculture species**. Immunological reagents to be developed through this grant include monoclonal antibodies (mAb) and polyclonal antibodies that identify the major leukocyte subpopulations (T and B lymphocytes, NK cells, neutrophils, macrophages, and dendritic cells) for many animal species including fish. In addition, recombinant cytokines and chemokines as well as antibodies to them and to their receptors, will be developed and these immune reagents will be valuable in research 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, and (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. Until recently, only a limited number of immune regulator and effector genes and their encoded polypeptides were identified in avian due to the low level of sequence homologies with their mammalian counterparts

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, blocking function and signaling, flow cytometric analysis, as well as for immunochemistry 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 current progress made in the development of immunological reagent for poultry only.**

## METHODS

### Cytokines

-15 genes of chicken **cytokines** (**IL-2**, IL-15, **IL-16**, IL-17a, IL-17d, IFN- $\gamma$ , TNFSF15 (TL1A), LITAF, **IL-10**, IL-4, IL-2 $\gamma$ , **IL-18**, LT, IL-12p35, and IL-12p40), and 4 **chemokines** (CCL4, CCL20, CXCR4, MIF) have been cloned and their full-length genes have been sent to Kingfisher Biotechnology Laboratory for production of recombinant proteins in *Pichia pastoris* expression system.

-Their functional activities were measured in bioassays using chicken primary macrophages and lymphocytes.

### Monoclonal antibody

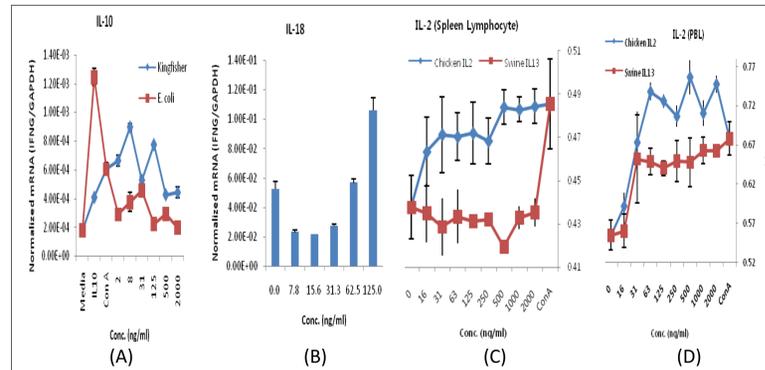
- Monoclonal Ab production

- Initial screening using ELISA against recombinant chicken cytokines -> single cell cloning of these hybridomas -> Western blot analysis -> Measuring bioactivity using FACS

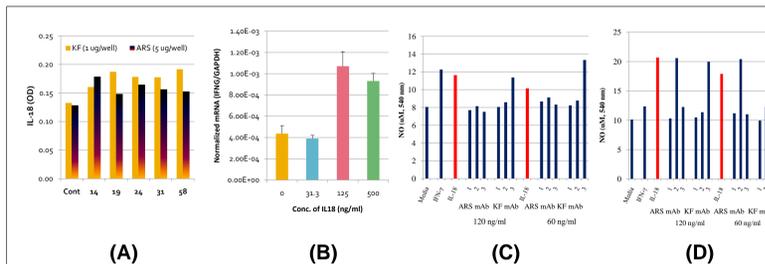
**Table 1. Cytokine genes cloned and sequenced in Dr. Lillehoj's Lab for Ab production (2006-2009)**

Gene	Vector	Nucleotide Accession #	Size (ORF)	Gene	Vector	Nucleotide Accession #	Size (ORF)
IL-2	pcDNA	AF017645	432 bp	CD86	pCR2.1	NM_001037839	852 bp
IL-15	pcDNA3	NM_204571	564 bp	IL-1 beta	pcDNA1	Y15006	804 bp
IL-16	pcDNA3	AJ508678	1824 bp	IL-2 receptor $\gamma$	pcDNA3	NM_204596	636 bp
IL-17	pSPORT6	AJ493595	510 bp	IL-18	pcDNA3	AJ277865	597 bp
IFN $\gamma$	puc18	AH009942	481 bp	Lymphotactin	pcDNA3	AF006742	294 bp
TNFSF15	pET32a	NM_001024578	720 bp	CCL4 (MIP-1 $\beta$ )	pBluescript-SK	NM_001030360	273 bp
NK-lysin	pET32a	DQ186291	423bp	CCL20 (MIP-3 $\alpha$ )	pET32a	NM_204438	303 bp
LITAF	pET32a	AY765397	447 bp	CXCR4	pcDNA3	NM_204617	1077 bp
IL-4	pET32a	NM_001007079	411 bp	IL-12p35	pET32a	NM_213588	618 bp
IL-10	pET32a	NM_001004414	528 bp	IL-12p40	pET32a	AY262752	948 bp
CD80	pCR2.1	NM_001079739	951 bp	IL-17D	pET32a	EF570583	351 bp
CD83	pCR.2.1	XM_418929	648 bp				

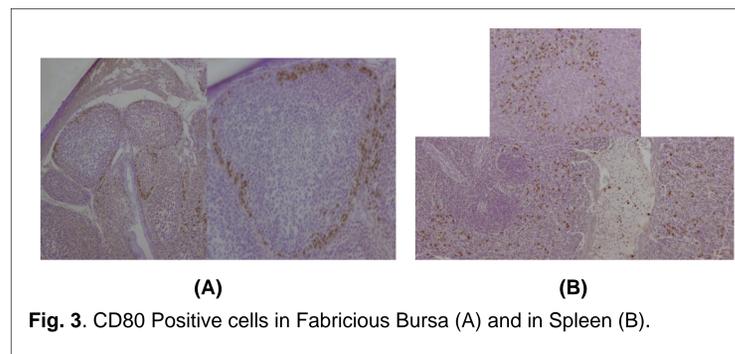
## PROGRESSIVE RESULTS



**Fig.1.** Bioassays of recombinant cIL-10 (A), cIL-18 (B), and cIL-2 (C, D). IFN- $\gamma$  transcript expression was measured following stimulation of chicken spleen lymphocytes with the serial dilutions of recombinant cytokines. *E. coli*- and yeast-expressed proteins were used for cIL-10 and cIL-18. Bioassays of recombinant cIL-2 (C,D) by cell proliferation assay. Chicken spleen and PBL blast cells were cultured with the serial dilutions of recombinant IL-2. Swine IL-13 was used as a negative control.



**Fig. 2.** Kingfisher (KF) mouse mAb response against rIL-18 produced by KF and ARS (A). Expression of IFN-gamma in splenocytes cultured with IL-18 for 24 hours (B). NO produced in HD11 cells after 48 hours of incubation with supernatants of SPL cultured with IL-18 and mAb for 24 hours (C). NO produced in HD11 cells after 72 hours of incubation with supernatants of SPL cultured with IL-18 and mAb for 72 hours (D).



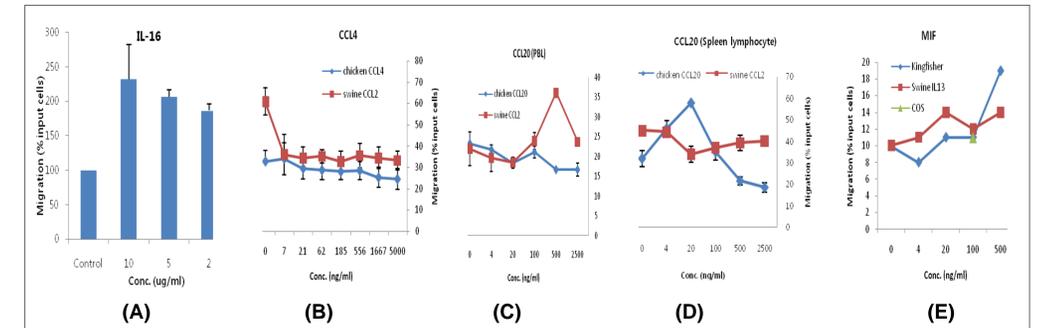
**Fig. 3.** CD80 Positive cells in Fabricious Bursa (A) and in Spleen (B).

**Table 2. Commercially available reagents and Abs for poultry**

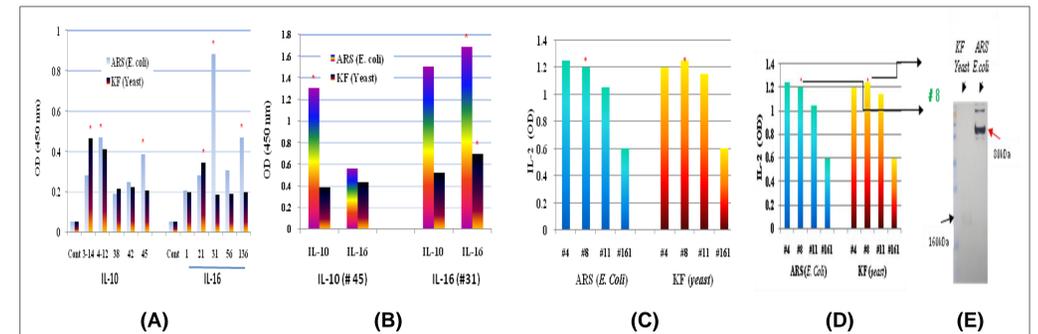
Protein or Ab *	Details
IL2, IL-10, IL-16, IL-18, CCL4, CCL20, MIF	Recombinant protein in <i>Yeast</i>
IL-16	polyclonal Ab

\* Accessibility and related information:

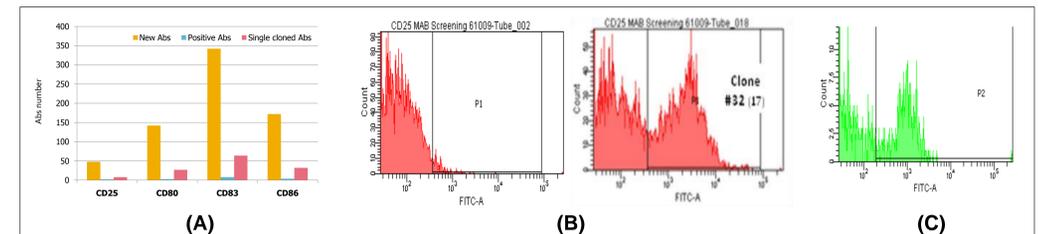
- Commercially available, accessible at cost  
- Kingfisher Biotech Inc.



**Fig. 4.** Chemotaxis bioassays for recombinant cIL-16 (A), cCCL4 (B), cCCL20 (C), and cMIF (D). Chicken SPL or PBL were cultured with recombinant proteins. Both chicken macrophage cell line (HD11) and PBMC were used for CCL4 (B) and MIF (E) assays respectively. Swine CCL2 and IL-13 were used as controls.



**Fig. 5.** IL-10 and IL-16 showed preferential binding to its respective immunizing antigen (A). Molecular weight of cIL-10 and cIL-16 was about 40kDa (B). Mouse mAbs production against recombinant IL-2 and IL-18 was carried out at ARS and several stable hybridomas were selected and cloned (C, D, E).



**Fig. 6.** Monoclonal Abs CD25, CD80, CD83, and CD86 against recombinant chicken cytokines tested and single cloned (A). Examples of mAb specifically recognizing chicken CD25 antigen (B) and CD80 antigen (C).

## PUBLICATIONS

- 2006:** Hong, Y.H., Lillehoj, H.S., Lee, S.H., Park, D.W., Lillehoj, E.P. 2006. Molecular cloning and characterization of chicken polysaccharide-induced TNF- $\alpha$  factor (LITAF). *Dev Comp Immunol.* 30: 919-929.
- 2007:** Park, SS, Lillehoj HS, Hong YH, Lee SH. 2007. Functional characterization of tumor necrosis factor superfamily 15 (TNFSF15) induced by lipopolysaccharides and *Eimeria* infection. *Dev Comp Immunol.* 31:934-944.
- 2008:** Hong, Y.H., Lillehoj, H.S., Park, D. W., Lee, S. H., Han, J. Y., Shin, J. H., Park, S. M., and Kim, J. K. 2008. Cloning and functional characterization of chicken proinflammatory cytokine IL-17D. *Veterinary Immunol Immunopathol.* 126:1-8.
- 2009:** Yoo, J.M., Jang S.I., Kim, S., Cho, J.H., Lee, H.G., Rhee, J.M., Lillehoj, H.S., and Min, W.G. 2009. Molecular characterization of duck interleukin-17. *Veterinary Immunol Immunopathol.* 132: 318-322.

## ACKNOWLEDGMENTS

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