US VETERINARY IMMUNE REAGENT NETWORK: PRIORITIZATION & PROGRESS.

J. Lunney[1], C. Baldwin[2], S.J. Black[2], P. Boyd[1], Dante Zarlenga[1], H. Lillehoj[1], J. LaBresh[3], D. Horohov[4], J. Hansen[5], N. Miller[6], E. Bengten[6], G. Chinchar[6], M. Wilson[6], and B. Wagner[7]

[1]USDA, ARS, BARC, Beltsville, MD USA, [2]University of Massachusetts, Amherst, MA USA, [3]Kingfisher Biotech, Minneapolis, MN USA, [4]University of Kentucky, Lexington, KY USA, [5]Western Fisheries Res Ctr USGS, Seattle, WA USA, [6]University of Mississippi Medical Center, Jackson, MS USA, [7]Cornell University, Ithaca, NY USA

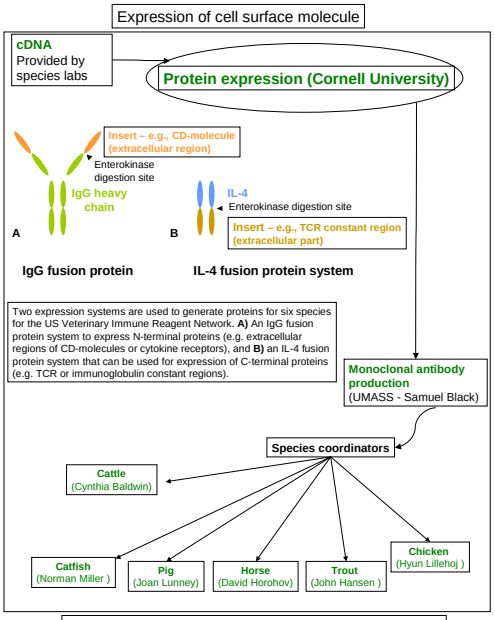
Abstract

The US Veterinary Immune Reagent Network (VIRN) (http://www.umass.edu/vetimm/) represents a broad community plan to begin to systematically address the immunological reagent gap for the US veterinary immunology research community including for the following groups: ruminants (concentrating on cattle), swine, poultry (primarily chickens), horses and aquaculture species (concentrating on channel catfish and trout) with a goal of developing 20 reagents per species group. Advances in genomics, including full genome sequences for chicken, cattle and horses, have provided improved data for gene and protein expression. The genome and EST data provides an excellent basis on which to accurately predict protein sequences for expression work that is the basis for production of needed immunological reagents including recombinant cytokines and chemokines and immune cell surface markers. Monoclonal antibodies (mAb) will be generated to identify the major leukocyte subsets (T and B lymphocytes, NK cells, macrophages, dendritic cells, neutrophils), react with cytokines/chemokines and their receptors, and target other important receptors that modulate immune function such as toll-like receptors are used to evaluate changes during disease including the causes of immune-pathology. These reagents will help scientists evaluate host responses to vaccination and provide the means to manipulate or modulate immune responses either to enhance protective immune responses to infections or to reduce immune-system-mediated pathology. The project directors are coordinating their efforts with other international groups and are continually revising the prioritization list and seeking input from scientists working with these species. A list of currently targeted reagents and progress regarding these will be presented.

U.S. Veterinary Immune Reagent Network Team

Overall Background: This is a multi-species immune reagent grant from USDA CSREES for development of a US Veterinary Immunological Reagents Network, which will support immunological reagents specific for ruminants, swine, poultry, equine and aquaculture species to advance veterinary immunology and disease control.

Expected Applications: These reagents will be used to: (1) evaluate changes during disease and following vaccination; (2) give scientists the ability to manipulate these cell populations to evaluate their roles in protective immunity as well as in immunopathology; and (3) begin to address the need for proteomics reagents.



Current results for Cell Surface Protein Expression

Current status	Cattle	Pig	Horse	Chicken	Catfish	Trout
Gene received	IL-23R			CXCR4		
Cloning into expression vector	IL-10Rβ	TCRα TCRβ	IgD TCRα	IL-2Rα	TCRβ TCRδ	TCRA TCRB TCRG
Stable transfectant		IL-4Rα IL-13Rα1	CD28 CD25			CD4 CD8
Protein purification	TCRδ		CD23			CD8
Send to UMass for hybridoma	TCRγ		CD40		TCRα TCRγ	

Members of the U.S. Veterinary Immune Reagent Network Team

(In order from left to right):

Dr. Joan Lunney (USDA ARS BARC; swine), Dr. Peter Johnson (USDA CSREES), Dr. Bettina Wagner (Cornell; equine and expression), Dr. Cyril Gay (USDA ARS), Dr. Cynthia Baldwin (Univ. Massachusetts; PI, bovine, hybridomas), Dr. Calvin Keeler (Univ. Delaware; chicken), Joanna LaBresh (Kingfisher; expression), Dr. David Horohov (Univ. Kentucky, equine), Dr. Norman Miller (Univ. Mississippi; catfish), Dr. John Hansen (USGS, trout).

Acknowledgements

Supported by the USDA-CSREES National Research Initiative grant for the U.S. Veterinary Immune Reagent Network and by ARS funds.

The authors thank their Toolkit collaborators for their expertise and helpful suggestions for these studies.

US Veterinary Immune Reagent Network Purpose:

Immunological reagents will be produced including cytokines and chemokines. Monoclonal antibodies (mAb) will be developed and targeted at identifying the major leukocyte subsets (T and B lymphocytes, NK cells, macrophages, dendritic cells, neutrophils), reacting with cytokines/chemokines and their receptors, and modulating immune function, such as toll-like receptors and regulatory signals. They will be used to evaluate changes during vaccine and disease responses, including identifying the causes of immune-pathology. Finally, these reagents provide the means to manipulate or modulate immune responses either to enhance protective immune responses to vaccines or to reduce immune-system-mediated pathology.

A broad community effort began in the US 24 months ago with the target species ruminants including cattle and sheep, swine, poultry including chickens and turkeys, horses, catfish, and trout. The project directors are coordinating their efforts with other international groups and are continually revising the prioritization list and seeking input from scientists working with these species.

Protein Expression:

Kingfisher Biotech, Joanna LaBresh, (soluble proteins)

Chemokines and cytokines will be expressed in yeast at Kingfisher, tested for bioactivity at species labs, and used for mAb production.

Cornell University, Bettina Wagner, (cell surface molecules)

Cell surface molecules fused to IgG1 or IL-4 for expression, tested for bioactivity at species labs, and used for mAb production
(See box below for more detailed information.)

Monoclonal Antibody Production:

University of Massachusetts, Amherst, Samuel Black,

- **Mice immunized with relevant protein and CFA or Gerbu adjuvant.**
- **Boost with IFA or Gerbu adjuvant.**
- **Adoptive transfer to naïve mouse with antigen.**
- **Fused at 3 days with thymocyte feeders in methylcellulose**

Ruminants:

Cynthia Baldwin, University of Massachusetts, Amherst

Bovine Priority List		
α IL-2	α IL-1	r IL-8
α TCRβ	α IL-4R	R IL-1β
α TCRα	α IL-12R	R IL-6
α IL-10R	α IL-23	r TNF-α
α CCR7	α CD151	α CCR2
α IL-4	α DC-SIGN	α CXCR3
α IL-12	α TLR4	α CCR5
α IL-6	α IL-23R	α CCR6
α IL-8	r IL-23	α IL-1β
α IL-10	α CD151	α TNF-α

Poultry:

Hyun Lillehoj, USDA-ARS (Maryland)

Poultry Priority List		
a Il-6	r IL-15	a CXCRI
a IL-1	a IL-16	a CXCR4
a IL-8	r IL-16	a CD40L
a IL-2	a IL-17	a CD80
a IL-15	r IL-17	a TLR 1
r IL-1	a IL-18	a TLR 2
a TGF-b	r IL-18	a TLR 3
r TGF-b	a IL-6R (CD126)	a iNOS
a IL-8	a IL-15Ra	a IL-10
r IL-8	a IL-2Rg	

Equine:

David Horohov, University of Kentucky, Lexington

Equine Priority List		
Il-1α	IL-13	TGFβ
IL-1β	IL-15	GM-CSF
IL-2	IL-17	CXCL9 (MIG)
IL-4	IL-18	CXCL10 (IP-10)
IL-5	IL-21	CXCL11 (I-TAC)
IL-6	IL-23	CCL2 (MCP1)
IL-7	IFN-γ	CCL3 (MIP-1α)
IL-8	IFN-α (family of 15)	CCL5 (RANTES)
IL-10	IFN-β	CCL11 (eotaxin)
IL-12 p35 & p40	TNF-α	VEGF-A or B

Catfish:

Norman Miller, Eva Bengten, Gregory Chinchar, Melanie Wilson, Univ. Mississippi Medical Center

Catfish Priority List	
α TCRa	α CD35z
α TCRb	α DAP10/12
α TCRg	α FceRg
α IgD	α perforin
non-immune IFN	α granzymes
α LITRs	α NK lysins
α IFN-2/ rIFN-2	α chemokines
α TNF/ rTNF	α chemokine receptors
α MHC II	α iNOS
α MHC I	α CD4
α b2M	α CD8
α FasR	α IL-1
α FasL	α B7
α FcR	

Trout:

John Hansen, Western Fisheries Research Center, USGS, Seattle

Trout Priority List	
α- TCRA	α- TdT
α- TCRB	α-CD45R
α- TCRG	α-CD79A/B
α- CD3	α- IL-8
α- CD28	α-BLIMP
α-CD4	CXCR3
α-CD4REL	α-Pax5
α-CD8A	CXCR9
α- IFNG	α-TLR3
α-TNF-α	α-TLR4
α- IFNA.2	α-TLR5
α- IgD	α-MHC class IA
α- IgT	α-MHC class IIB

Swine Priority List: Joan Lunney, Pat Boyd, USDA ARS, BARC, Maryland

The emphases for swine will be on developing and characterizing bioactive immune proteins, cloned cytokine and chemokine proteins, as well as monoclonal antibodies (mAbs) to these proteins and their receptors. Additional mAb will be produced to swine cell subset proteins, the CD antigens, and toll-like receptor (TLR) proteins. Examples of our current approaches for each type of reagent is given on this poster as well as the current priority list. A separate anti-Ig effort for swine is underway with Drs. J. Butler, Univ. IA, and S. Muyldermans, Flanders Univ., Belgium, with US National Pork Board funding.

Summary of Current Swine VIRN Toolkit Plans for reagent Development

	Type Reagent	Common Name	Gene Symbol	Swine Unigene clone	U Mass - Clone and Sequence	Protein expression	mAb Production	Planned Bioassay
1	cell surface	TCR alpha	TRA@	L21158	U completed	C in process		FCM
2	cell surface	TCR beta	TRB@	AB079521	U completed	C in process		FCM
3	cell surface	CD45RO	PTPRC	AY444871	peptide*	N/A	7/16/2007	CD45 cell based ELISA
4	cell surface	CD213A1	IL13Ra1	AY266142	plasmid**	C stable expression		FCM
5	cell surface	CD124	IL4Ra	AY266143	plasmid**	C stable expression		FCM
6	cytokine	IL-13	IL13	NM_213803	U completed	K in process		IL-6 production
7	cytokine	IL-15	IL15	U58142	U completed	K in process		CTLL-2
8	cytokine	IL-7	IL 7	AB035380	U completed	K in process		T cell proliferation
9	cytokine	CTLA-8	IL17	AB040441	U completed	K in process		IL-6 production
10	chemokine	MCP-1	CCL2	X79416	U completed	K in process		Chemotaxis
11	chemokine	IP-10	CXCL10	AY789646	U completed	K in process		Chemotaxis
12	chemokine	MIP-1a	CCL3L1	AY643423	U completed	K in process		Chemotaxis
13	chemokine	RANTES	CCL5	AJ583704	U completed	K in process		Chemotaxis
14	chemokine	MIG	CXCL9	BP169836	U completed	K in process		Chemotaxis
15	chemokine	IP-9	CXCL11	BX914688	B in process			Chemotaxis
16	cytokine	IFNB	IFNB1	AY687281	B in process			antiviral activity
17	cytokine	IFNa	IFN1@	AY345969	U completed	K in process		antiviral activity
18	cell surface	CD197	CCR7	AB090356	B in process	N/A		FCM
19	cell surface	CD183 &182	CXCR3	AJ851240	B in process	N/A		FCM
20	cell surface	CD127	IL7Ra	BP157102	B in process	N/A		FCM
21	cell surface	CD101	IGSF2		B in process	N/A		FCM

B = BARC in silico gene discovery; cDNA preparation, primer design for U Mass

U = U Mass cloning into appropriate vector; confirmatory sequencing

C = Cornell mammalian stable expression with equine IgG or IL4 expression vectors

K = Kingfisher *Pichia* expression

* CD45RO peptide designed with, and transfected cells for ELISA provided by, W Schnitzlein and F Zuckerman-U IL

** Plasmid provided by D Zarlenga, BARC

FCM = Flow cytometry on T cells/PBMCs