

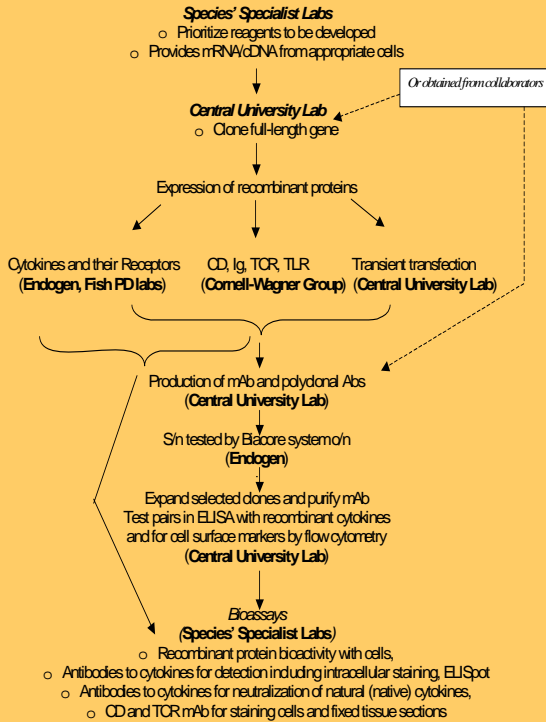
US Veterinary Immune Reagent Network



Abstract

As stated at USDA <http://www.csrees.usda.gov>, "a major obstacle to advances in veterinary immunology and disease control is the lack of sufficient immunological reagents specific for ruminants, swine, poultry, equine and aquaculture species". Sets of reagents, i.e., monoclonal (mAb) and polyclonal antibodies, that can identify the major leukocyte subsets (T and B lymphocytes, NK cells, macrophages, dendritic cells, neutrophils) are needed to evaluate changes during disease and following vaccination and to give scientists the ability to manipulate these cell populations in order to evaluate their roles in protective immunity as well as in immunopathology. In addition, it is crucial to have recombinant cytokines and chemokines and antibodies to them and their receptors to understand their contributions to inflammation and protective immunity. Reagents to identify immunoglobulin (Ig) isotypes are needed since antibody isotypes differ from one another functionally and, thus, with regard to their effect on disease outcome. Development of the above reagents will address the USDA/NRI goal of enhancing the safety of the Nation's agriculture and food supply by aiding in the development of vaccines. This proposal presents 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 with some evaluation of reagents on turkey cells), horses and aquaculture species (concentrating on channel catfish and salmonid trout, two of the principal economically important species) with a goal of 20 reagents per species group. The reagents produced will include bioactive recombinant cytokines and chemokine proteins, expressed using mammalian cells, *Pichia pastoris* or *E. coli* systems, as well as antibodies and mAb to them, their receptors, as well as mAb to Ig isotypes, T cell receptors (TCR), Toll like receptors (TLR) and other CD molecules. Our goal is to produce antibodies that function in ELISA and ELISpot assays, for intracellular staining, for blocking function and signaling, and that are useful in flow cytometric applications as well as in fixed tissue sections. Products developed in this proposal will benefit a large group of researchers including veterinary immunologists, pathologists and microbiologists.

Flow Chart of the Systematic Centralized Approach



PROJECT DIRECTORS

- Cynthia Baldwin, University of Massachusetts** cbaldwin@vascl.umass.edu
 – Ruminants bioassays & Gene Cloning & Antibodies
- John Hansen, WFRG-USGS-Biological Resources Division** jhanzen@usgs.gov
 – Trout bioassays, genes & recombinant proteins
- Dave Horohov, University of Kentucky** david.horohov@uky.edu
 – Horse bioassays
- Joanna LaBresh, J.LABRESH@COMCAST.NET**
 – Recombinant mammalian/avian cytokines/receptors
- Hyun Lillehoj, USDA-ARS Beltsville** hillehoj@anrl.barc.usda.gov
 – Poultry bioassays & genes
- Joan Lunney, USDA-ARS Beltsville** jlunney@anrl.barc.usda.gov
 – Swine bioassays & genes
- N.Miller, Wilsong, Bengten, Chinchar, Univ Mississippi** nmiller@microbio.usm.edu
 – Catfish bioassays, genes & recombinant proteins
- Bettina Wagner, Cornell University** bw73@cornell.edu
 – Recombinant membrane-bound glycoproteins

Types of Reagents Targeted

- > Cytokines bioactive recombinant proteins as well as antibodies to them
- > Antibodies
 - o Cytokine receptors
 - o Immunoglobulin isotypes
 - o T cell receptor constant and variable regions
 - o Co-receptors that affect hematopoietic functions such as FcR, TLR, MHC II family members
- > Antibodies with the following properties
 - o For cytokines
 - o mAb pairs for ELISA and ELISpot assays (i.e., mAb that react with 2 different epitopes on same molecule) for cytokines
 - o Single mAb for intracellular staining for cytokines
 - o Binding antibodies for receptors and co-receptors
 - o For cytokines
 - o mAb pairs for ELISA and ELISpot assays (i.e., mAb that react with 2 different epitopes on same molecule) for cytokines
 - o Single mAb for intracellular staining for cytokines
 - o Binding antibodies for receptors and co-receptors
 - o mAb pairs for subunit differentiation
 - o Separate mAb for flow cytometry with living cells only
 - o React in fixed tissue sections
 - o Western blotting

Minimum Deliverables Projected

REAGENT TYPE	Swine	Cattle	Horse	Chicken	Trout	Catfish
Bioactive proteins & used for generating mAb (LaBresh)	10	10	10	15	0	0
Recombinant proteins small quantities for mAb (10-15/yr X 3 yrs) (Cornell)	5	5	18	5	0	0
mAb production (24/yr X 3 yrs = 72 total) (Univ Massachusetts)	12	12	12	12	12	12
Recombinant proteins -Species fish labs	0	0	0	0	10	10
Estimated total deliverables by species groups	22	22	32	27	12	12

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PRELIMINARY PRIORITIZATION BASED ON SURVEY RESULTS

Responses are not necessarily in priority order for each species, from preliminary surveys completed by species PIs, as time permitted while developing this proposal, are noted.

*Ab to molecule indicated; **recombinant protein; ***BBSRC will target; () being made

Species	Catfish	Cattle	Chicken**	Horses	Swine**	Trout
1	αTCR α	αIL-2	αIL-6	αIL-5	αIL-13	αIFN-γ
2	αTCR β	αTCR β	αIL-1	αIL-10	αTNF-α	αIFN-α
3	αTCR γ	αTCR α	αIL-8	αIL-12	αTCR α	αTCR β
4	αIgM	αIL-10R	αIL-2	αIL-13	αTCR β	αCD1
5	Non-immune IFN	αCCR7	αIL-15	αIL-27	αIL-5	αCD28
6	αLITRs	αIL-4	αIL-1	αIFN-γ	αCD45RB	αCXCR9
7	αIFN-2	αIL-12	αIFN-β	αIFN-β	αTLR4	αCXCR3
8	αTNF	αIL-6	αIFN-β	αIFN-β	αCXCL9	αCD4
9	αMHC II	αIL-8	αIL-8	αIL-10	αCXCR3	αCD8
10	αMHC I	αIL-10	αIL-8	αIFN-γ	αIL-23	αTLR3
11	αβM	αIL-1	αIL-15	α(GDF11)	αCD1-23R	αTLR5
12	αFasR	αIL-4R	αIL-15	αIL-4	αCD11b	αCD45R
13	αFasL	αIL-10R	αIL-16		αCD45RO	αIg subclasses (IgD & IgT)
14	αFcR	αIL-12R	αIL-16	αIL-12	αCD45RB	αMHC class IA
15	αCD3ε	αIL-23	αIL-17	αIL-13	αCD20	αMHC class IIAB
16	αDAP10/12	αCD151	αIL-17	αIL-27	αCD34	αIL-1β
17	αFcRγ	αDC-SIGN	αIL-18	αIL-8	αCD151	αCD79A/B
18	α perforin	αTLR2	αIL-18	T1 X Ig	αCCR7	αBLIMP
19	α granzymes	αTLR4	αIL-6R (CD126)	αIgD	αCXCL10	αPax5
20	αNK lysins	αIL-23R	αIL-15Rα	αIgG2	αCCL2	αTNF-α
21	α chemokines	αIL-2R	αIL-2Rγ	αIgG3	αCCL5	PNV marker
22	α Chemokine receptors	αIL-8	αCXCR1	αIgG7		
23	α MDS	αIL-1β	αCXCR4	αCD19		
24	α CD4	αIL-6	αCD40L	αCD21		
25	α CD8	αTNF-α	αCD80	αTCRα/β		
26	α IL-1	αCCR2	αTLR1	αTCRγ/δ		
27	α B7	αCXCR3	αTLR2	αCD14		
28	αCCR5	αTLR3	αCD23	αCD23		
29	αCCR6	αCXCR5	αCCR6	αCCR6		
30	αIL-6	αIL-10	αCD25			
31	αIL-1β		αCD80			
32	αTNF-α		αCD86			

COLLABORATORS

Species expertise	Individual and affiliation
catfish	Mark Jahn, Montana State University
catfish	Susan Fisher, ARS-USDA Purdue University
catfish	Ronald Thune, Louisiana State University
catfish	Wendy Brown, Washington State University
catfish & swine	Doug Bannerman, USDA ARS, BARC, MD
chicken	Calvin Keeler, University of Delaware
chicken	Darell Kapczynski, USDA ARS Scientist, SEPR1, GA
chicken	Uma Babu, US-FDA, MD
chicken	Thomas Scott, Clemson University
chicken	Douglas Antezok, Cornell University
chicken	James Beknap, Ohio State University
chicken	Steve Hines, Washington State University
chicken	Jeff Stott, Univ. California Davis
chicken	Samuel Black, University of Massachusetts
chicken	Fredrico Zuckerman, University of Illinois
chicken	William Golde, USDA ARS, Plum Island, NY
chicken	Steve Kaattari, East Carolina University
chicken	Charles Rice, Clemson University
chicken	Ching Chung Wu, Purdue University
chicken	Philipp Klees, USDA-ARS Auburn
chicken	Geoff Waldbauer, USDA-ARS Catfish Genetics Group
chicken	Gregory Watt, Medical University of South Carolina

CONSULTANTS

Species expertise	Individual and affiliation
catfish	Mark Jahn, Montana State University
catfish	Susan Fisher, ARS-USDA Purdue University
catfish	D. Mark Estes, Univ. Texas Med. Br. Galveston
catfish	Andrzej Kasib, University of Guelph, Canada
catfish & swine	Dante Zerbe & Harry Dawson, USDA ARS, Beltsville
chicken	Kirk Klasing, University of California Davis
chicken	Narayan Rath, USDA ARS, Arkansas
chicken	John Butler, University of Iowa
chicken	Darell Kapczynski, USDA ARS Scientist, SEPR1, GA
chicken	Uma Babu, US-FDA, MD
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chicken	Philipp Klees, USDA-ARS Auburn
chicken	Geoff Waldbauer, USDA-ARS Catfish Genetics Group
chicken	Gregory Watt, Medical University of South Carolina

Advisory/Oversight Board

ROLE	INDIVIDUAL	AFFILIATION
Catfish	Mr. Hugh Warren	Executive Vice President, Catfish Farmers of America
Stakeholders	Dr. John Adams	National Milk Producers Federation
Cattle Stakeholders	Dr. Janet Fulton	Hy-Line International
Chicken	Dr. Janet Adams	
Stakeholders	Mr. Gene Pranzo	President of Dorothy Havemeyer Foundation
Horse Stakeholders	Dr. Paul Sundberg	Vice President Science & Technology, National Pork Board
Swine Stakeholders	Dr. Scott LaPatra	Director Research & Development, Clear Springs Foods
Trout Stakeholders	Dr. Susan Lamont	Iowa State University
Species - chicken	Dr. Michael Murtaugh	University of Minnesota; coordinator of USDA multi-investigator PSSRV grant
Species-swine	Dr. Greg Wiens	USDA ARS, National Center for Cool and Cold Water Aquaculture, Kearneysville, WV
Species-trout	Professor William Davis	VMRD, Pullman, WA; Coordinating Immunology section of USDA multi-investigator John's Disease Grant
Veterinary Reagent Industry	Dr. Jim Kaufman	Project Director for UK Immunological Toolbox (BBSRC) initiative, Compton, England
International	Dr. Edwin Tijhaar	Director of the Dutch initiative "The Cytokine Center, www.cytocen.com, Utrecht, The Netherlands
International	Dr. Gary Etrican	Chair, International Union of Immunological Societies Veterinary Toolkit Committee, Edinburgh, Scotland (Committee mandated to inventory veterinary reagents and prioritize need)
International	Dr. John Lowenthal	Coordinator of reagents for swine and poultry in Australia, CSIRO, Brisbane, Australia
International	Dr. Chris Seombes	Leader in trout cytokine gene identification and expression, Scotland, UK
Reagent Initiative	Dr. Cyril Gay	ARS National Program Leader, Animal Health
USDA-ARS	Dr. Robert Heckert	ARS National Program Leader, Exotic & Emerging Diseases