US Veterinary Immune Reagent Network - Progress report for *all species*

(*www.vetimm.org*)

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ABSTRACT

The gene cloning phase of the project was completed during the early part of Year 3 (Spring 2008). In total, the Network cloned more than 150 complete expressed sequences of genes for cytokines, chemokines and cell surface markers of hematopoietic cells of cattle, horses, pigs, trout, catfish and chickens. The genes for chemokines and cytokines are being expressed at Kingfisher Biotech (KF) in yeast and in mammalian cells at Cornell for cell surface molecules as well as in the fish labs in bacteria or mammalian cells. Many of these cytokines and chemokines have been expressed and assessment of bioactivity of chemokines and cytokines has been underway during Year 3-4 in the species labs for bovine, equine, chicken and swine . Fish cytokines are being produced starting in early 2009 . Cell surface molecule expression has continued since Year 1 and will be completed in Year 4. Generation of mAb to those cell surface molecules (and to the chemokines and cytokines selected) was made good progress during Year 3 and is continuing through Year 4 .

BACKGROUND

Additional information of the progress is summarized by species at *www.vetimm.org* on the various web pages and in attached PDFs on the web page as FASTA files, alignments of genes being expressed, commercially available reagents for the species, protocols for bioassays of cytokines and chemokines, primary references for the gene cloning and cytokine detection for each species.

Chemokines & Cytokines Following the completion of the gene cloning phase for the cytokines and chemokines, a large scale alignment and annotation of the expressed sequences was done to confirm the correctness of the sequences before expression at KF. These are shown on the website. Whole sequence included signal/leader sequence while those designated as KF are ready for expression in the yeast system in most cases without signal sequence or pro-peptides.

- **All these genes are available for distribution to the scientific community upon request and we are investigating feasibility of deposition in gene banks.**
- **Virtually all sequences have been deposited in GenBank.**
- **Kingfisher (KF) biotech is in the process of expressing all these in *Pichia* and those expressed have been sent the various species coordinators for biotesting. This began in Feb 2008.**
- **The bioactive proteins are commercially available (see Kingfisher website www.KingfisherBiotech.com) and a limited amount directly distributed to scientists.**
- **A selected number will be used for producing mAbs by US-VIRN.**

Cell Surface Molecules These genes were largely cloned by US-VIRN and are being transfected into CHO cells at Cornell by Bettina Wagner while the fish labs have produced their cell surface proteins in bacteria or mammalian cells; All will used for production of MAb.

- **Gene sequences have been/will be deposited into GenBank.**
- **Most genes are available upon request.**
- **All the proteins in this category have been, are in the process of or will be used for producing mAbs.**
- **Transfected cell lines will also be available for testing other potential cross-reactive mAbs.**

Monoclonal antibodies The cell surface molecules being expressed at Cornell in the Wagner Lab or in the Hansen or Mississippi fish labs will all be used for producing monoclonal antibodies (mAb) at UMass and Cornell or in the fish labs. In addition, a limited number of mAb will be made in this round against cytokines or chemokines that have shown bioactivity in the Species labs.

- **These are being continually re-prioritized by the species coordinators in conjunction with the scientists in field but the current version is shown.**
- **The hybridoma cell lines that produce the mAbs will be deposited in cell banks for distribution upon request and distributed to a limited number of scientists directly by Network members; the mAb they produce will available through commercial companies including Kingfisher.**

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CYTOKINES & CHEMOKINES: Gene expression, bioactivity and targeting for mAb

Immune Marker	US-VIRN's clone's GenBank #	√ = Transformed in expression vector for yeast	√ = Expressed in yeast (OR expected - date noted)	√ = Purified on FPLC (OR expected - date noted)	Bioactivity testing	Monoclonal antibodies produced (blank = not this round)
CATFISH						
IFNγ 2B	DQ124250	√	7/09			Will target
CATTLE						
CCL2	EU276069	√	√	√	Protein at UMass	Tested at Cornell with α-swine CCL2 mAb, some+
CCL5	EU276060	√	√	√	Protein at UMass	Will target: Protein at UMass
CCL11	EU744565	√	√	√		
CXCL9	EU276061	√	√	√	YES (UMass) but variable results	
CXCL10	EU276062	√	√	√		
CXCL11	EU276063	√	√	√	YES (UMass) but less than R&D's	
IFN-γ	EU276066	√	√	√	YES (UMass) low for 1 lot and negative for others stored incorrectly	Will target: Protein at UMass
IFN-α	EU276064	√	√	√	YES (UMass) low activity-new lot expressed in 2009?	Mice immunized, fusion 5/29/09
IFN-β	EU276065	√	√			
IL-1β	EU276067	√	low expresser, being retransformed and expressed			
IL-2	EU276068	√	√	√	YES on TF1 cells; YES (UMass) bovine PBMC	
IL-4	EU276069	√	√	√	YES on TF1 cells; YES (UMass) ConA-act'd cells	Will target: Protein at UMass
IL-5	EU276070	√	3/17/09 low expresser, being retransformed and expressed			
IL-6	EU276071	√	√	√	NO (UMass) on B9 cells as reported by others for bovine; working out Q-RT-PCR for IL-23 expression	Will target: protein at UMass
IL-7	EU276072	√	3/17/09 low expresser, being retransformed and expressed			
IL-8	EU276073	√	√	√	Protein at UMass	Sheep mAb made by UK Toolkit cross-react with our bovine IL-8
IL-10	EU276074	√	√			
IL-12p35	EU276075	√	√			
IL-12p40	EU276076	√	√	√		
IL-13	EU276077	√	√	√	YES on TF1 cells; YES (UMass) increased MHC class II expression on bovine PBMC	Fusion done 2x's at UMass; no mAbs produced
IL-15	EU682380	√	√	√	YES (UMass) on bovine PBMC had some activity at 300ng/ml but less than for human IL-15	
IL-17	EU682381	√	√	√	YES (UMass) increased mRNA for IL6 and IL8 at 48 and 72 hr	Will target: protein at UMass & UK
IL-18	EU276078	√	√	√		
IL-23A	EU616677	√	√	√	One chain expressed so won't be tested in bioassay	Fused, 5 weak mAbs in ELISA turned negative
TNF-α	EU276079	√	√	√	Protein at UMass	Will target: protein at UMass
CHICKEN						
CCL4	NM001030360	√	√	√	Yes(BARC:No activity: Will repeat)	Will target: protein at UMass
CCL20	NM204438	√	√	√	YES (BARC: showed activity on splenocytes: Will repeat)	
IFN-γ	AH009942	√	3/17/09 low expresser, being retransformed and expressed			mAb made, available at BARC
IL-1b	Y15006	√	3/17/09 - low expresser, being retransformed and expressed			mAb made, available at BARC
IL-2	AF017645		√	√	YES (BARC)	
IL-4	NM 001007079	√	1/20/09, 3/3/09, 3/31/09 low expresser, being retransformed and expressed			Will target
IL-10	NM 001004414	√	√	√	YES (BARC)	mAbs made (UMass), tested at BARC (Will repeat)
IL-12p35	NM213588	√	3/24/09 low expresser, being retransformed and expressed			
IL-12p40	Ay262752	√	6/09			
IL-15	NM 204571	√	3/17/09 low expresser, being retransformed and expressed			mAbs made, Available at BARC
IL-16	AJ508678	√	√	√	YES (BARC)(Will repeat)	MAbs made (UMass); tested at BARC(Will repeat)
IL-17A	AJ493595		3/3/09 low expresser, being retransformed and expressed			Will target
IL-17D	Ef570583	√	3/17/09 low expresser, being retransformed and expressed			Will target
IL-18	AJ277865	√	√	√	YES (BARC)	Fusion made (UMass) and clones selected – being tested at BARC
LITAF	AY765397	√	4/09 √ low expresser, being retransformed and expressed			
Lymphotactin	AF006742	√	6/09			
MIF	M95776	√	√	√	Protein at BARC	
TNFSF15 (TL1A)	NM001024578	√	3/24/09 low expresser, being retransformed and expressed			
HORSE						
CCL2	EU438774	√	√	√	NO (Cornell) did not induce basophil degranulation; Protein will be tested at UMass for migration assay	6 α-swine CCL2 mAb react with equine CCL2 and bovine CCL2
CCL3	EU438775	√	√	√	Protein at UMass	Immunization ongoing
CCL5	EU744564	√	√	√	Protein at UMass	Will target: Protein at Cornell
CCL11	In progress	√	√	√	Protein at UMass	Will target: Protein at Cornell
CXCL9	EU438776	√	√	√		Fusion performed at Cornell
CXCL10	EU438777	√	√	√		Immunization ongoing
GM-CSF	EU438778	√	√	√	Protein at Cornell	Fusion screened positive clones identified
IFN-g	U04050	√	low expresser, being retransformed and expressed			2 mAbs, working on additional protein to get better mAb variety
IFN-α1	EU682378	√	√	7/09		mAbs to IFN-a have been developed at Cornell in 2007 using mammalian expressed protein, Existing Luminex assay for IFN-a – will be tested soon for crossreactivity with bovine IFN-a
IL-1β	EU438767	√	√	√	Protein at Cornell	Will target: Protein at Cornell
IL-2	EU438768	√	√	√	YES (UKentucky) Protein at UMass	6 mAb made, Work in ELISA with mammalian expressed IL-2 and detected IL-2 by FACS
IL-4	EU438769	√	√	√	YES (UKentucky) Protein at UMass	4 mAb made, Luminex and flow analysis established
IL-5	U91947	√	3/10/09 low expresser, being retransformed and expressed			
IL-6	EU438770	√	√	√	YES, B9 cells	Fusion made at Cornell, weak clones, 2 mAbs will be grown up for further characterization – not the best mAbs
IL-10	EU438771	√	3/17/09 low expresser, being retransformed and expressed			mAbs made (Cornell), IL-10 Luminex assay and flow cytometric analysis established
IL-13	EF645663	√ being recloned				One fusion done using mammalian protein, needs repeating
IL-15	EU682379	√	√	√	Tested (UMass) on equine PBMC, no activity of KF but human IL-15 worked	Fusion done at Cornell, 2 mAbs identified for further characterization
IL-17	EU744563-no ss	√	2/10/09 low expresser, being retransformed and expressed			
IL-18	EU438772	√	6/09			
IL-23A	EU438773	√	6/09			
TGF-β	X99438	√	6/09			
TNF-α	EU438779	√	3/24/09 low expresser, being retransformed and expressed			
SWINE						
CCL2	EU682382	√	√	√	YES (BARC) positive chemotaxis for PBMC monocytes; increased ICAM on swine epithelial cells	12 mAb and 6 X-rx with equine CCL2, 5 with bovine CCL2
CCL3L1	EU364893	√	√	√	Protein at BARC	
CCL4	EU364894	√	√	√	YES (BARC) positive chemotaxis for PBMC monocytes	Will target: Protein at UMass
CCL5	EU44561	√	√	√	Protein at BARC, 1st screen + chemotaxis with PBMC monocytes	Will target: Protein at UMass
CXCL9	EU36897	√ cloning issue				
CXCL10	EU364898	√	√	√	YES (BARC) positive chemotaxis for PHA IL-2 stimulated T cells	Fusion done, 1 mAb; KF sending new cleaner batch for more mAb
CXCL11	EU682377	√	√	√	YES (BARC) positive chemotaxis for PHA IL-2 stimulated T cells	Fusion done, 1 mAb; KF sending new cleaner batch for more mAb
IL-7	EU364895	√	2/3/09 low expresser, being retransformed and expressed			Will target
IL-13	EU682383	√	√	√	Low activity onTF1 cells and in assay run at PIADC; Protein at BARC	Fused 3/9 and 3/23; KF sending new cleaner batch for more mAb
IL-15	NM 214390	√	√	√	YES (BARC) but less than human IL-15 or Invitrogen's SwIL-15; Golde limited/no NK cell restimulation	
IFN-α	EU364896	√	low expresser, being retransformed and expressed		NO (UMass) activity on MBDK cells	NO! they are already available
IFN-β1	EU744562	√	√	√	YES (UMass) MBDK cells; good activity relative to human IFN control	Fused at UMass; two candidate mAb
TNF-α	EU682384	√	6/09			
TROUT						
IFN1	AM489418	√	√	√		mAb in progress (UMass Dartmouth)
IFN2	AJ582754	√	√	√		mAb in progress (UMass Dartmouth)
IFN3	AM235738	√				Will target
IFN4	AJ616215	√	3/10/09 low expresser, being retransformed/ expressed			Will target
IFNγ		Will express at USGS				Will target

Hybridomas not planned

July 1, 2009 – Updates by all

CELL SURFACE MOLECULE EXPRESSION AND MAB PRODUCTION

Molecule	Full length cDNA with signal sequence GenBank#	Stable transfectant	Protein being produced/ Purified	mAb fusion status	mAb on recombinant protein	mAb reactivity on native protein
CATFISH						
TCR α	U58505	√	√	mAb lost, repeating		
TCR β	U39193	√				
TCR δ		√				
TCR γ	DQ435303	√	√	mAb made (UMass); lost activity	Yes	
IgD	U67437	√ (E.coli)	√	mAb made (UMass) & characterized	Yes	Yes
IgL lambda	EU872025	√ (E.coli)	√	mAb made (UMass)		
IgL sigma	EU872025	√ (E.coli)	√	mAb made (UMass)	Yes	Yes
MHC class II	U77597/U77598	√ (E.coli)	√	mAb characterized (UMiss)	Yes	Yes
CD45	AY366233	NA	NA	mAb made & characterized (UMiss)		Yes
CD4-1	DQ35301	√ (E.coli)	√	mAb made (UMass)	yes	yes
CD3γδ	FJ804169					
CD8β	FJ804170					
CD8α	GQ179649					
CD3ε						
CATTLE						
TCR δ		√	√	Mice immunized (UMass)		
TCRγ-C3	BC149622	√	√	mAb made (UMass)	yes	Several mAb that react within the TCR delta+ population
TCR α	D10394					
TCR β	D90139					
IL-23R	EU616678	√	No secretion, recloning			
IL-10R	BC123561	√	√	Mice immunized (UMass)		
CCR7	AY834253	√	√	Mice immunized (UMass)		
CHICKEN						
CD25	NM_204596	√	√	mAb made (UMass)	2 clones	In tesing
CXCR4	NM_989948	In progress				
CD80	NM001079739	√	√	Fused (UMass)	6 clones	In testing
CD83	XM418929	√	√	Mice immunized (Umass)		
CD86	NM001037839	√	√	Mice immunized (Umass)		
HORSE						
CD40	NM001081902	√	√	mAb made (UMass)		Multiple clones, characterization ongoing
CD23	NM001081807	√	√	mAb made (Cornell)	√ (Cornell)	7 mAbs; different epitope specificities, flow cytometry and Western blotting
CD25	EU184658	√	√	Done, repeating fusion (Cornell)		1 mAb became negative, repeat
CD28	EU000412	√	√	mAb made (Cornell)	√ (Cornell)	2 mAbs; detect T-cell subpopulation of equine PBMC
FcεRIα	submitted	√	√	mAb made (Cornell)	√ (Cornell)	2 mAbs; characterization ongoing
TCR α	Submitted	√				
TCR ß	Submitted	√				
TCR γ	Submitted	√				
CD14	AF200416	√	√	mAb made (Cornell)	√ (Cornell)	4 mAbs; different epitope specificities, flow cytometry and Western blotting
SWINE						
TCR α	EU364899	√	√	mAb with Ig fusion protein failed; repeating IL-4 fusion (Cornell)	- (Cornell)	No positive mAb (BARC) reactive with T cells
TCR β	EU364900	√	√	mAb with Ig fusion protein failed; repeating IL-4 fusion (Cornell)	- (Cornell)	No positive mAb (BARC) reactive with T cells
IL-4Rα	AY266143	√	√	mAb made (Cornell)	√ (Cornell)	Positive mAb (BARC) on lymph node cells; also on ATRA stimulated IPEC-J2 cells
IL-13Rα1	AY266142	√	√	mAb made (Cornell)	√ (Cornell)	Positive mAb (BARC) on IPEC-J2 cells
CD45RO	EST- F14696	-	Commercial peptide	Fusion made; repeating (UMass)	Several positive mAb (UMass)	No positive mAb (BARC) reactive with CD45RO+ CHO cells of PBMC older pigs
TROUT						
LCK	AY973032	√	√ (E.coli)	mAb made (USGS)	yes	Yes
CD3	CA357253	√	√	mAb in progress (UMass Dartmouth)		
CD4-REL	AY973029	√	√ (E.coli)	mAb in progress (UMass Dartmouth)		
CD4	AY973030	√	√ (E.coli & HEK293)	mAb in progress (UMass Dartmouth)		
CD8	AF178054	√	√			
CD28	AY789435	√	√ (E.coli & HEK293)			
CD83	AY263796	NA	√ (E.coli)			
CTLA-4	AY789436	√				
TCR A	EU072698	√				
TCR B	EU072699	√				
TCR G	EU072700	Not secreted, recloning				
IgL1	CAA46362	Peptide	√	mAb made (UMass Dartmouth)	Nd	Yes
IgL2	AAB41310	Peptide	√	mAb made (UMass Dartmouth)	Nd	Yes
IgL3	GQ217535	Peptide	√	mAb made (UMass Dartmouth)	Nd	yes
IgT $C_\gamma 2$		NA	√ (E.coli)	mAb in progress (UMass Dartmouth)		
IgT $C_\gamma 4$		NA	√ (E.coli)	mAb in progress (UMass Dartmouth)		

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