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

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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 **(Table 1)** and in mammalian cells at Cornell for cell surface molecules as well as in the fish labs in bacteria or mammalian cells **(Table 2)**. Many of these cytokines and chemokines have been expressed and assessment of bioactivity of chemokines and cytokines has been underway during Year 3 in the species labs for bovine, equine, chicken and swine **(see Table 1)**. Fish cytokines are being produced starting in early 2009 **(see schedule, Table 1)**. 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) has made good progress during Year 3 and will continue through year 4 **(Table 3)**. Additonal 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 (Table 1) 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 (see **Table 2**).

Cell Surface Molecules (Table 2) 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 (Table 3) 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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Table 2. CELL SURFACE MOLECULE PRODUCTION SCHEDULE

Expression in CHO cells: (√=done; Dates – when protein will ready for mAb production) Those expressed bacteria are indicated as such	Molecule	Gene cloned*	Gene in expression vector	Stable transfectant	Protein purification	MAb in progress or made
Pig						
√ (recloning May '09 into different vector)	TCRα					
√ (recloning May '09 into different vector)	TCRβ					
√	IL-4Rα					
√	IL-13Rα1					
√ (peptide)	CD45					
Horse						
√	CD40					
√	CD23					
√	CD25					
√	CD28					
√	FcRαR1					
July '09	IgD					
June '09	TCRα					
June '09	TCRδ					
√	TCRγ					
Bovine						
Feb '09	TCRδ					
	IL-23R					
March '09	IL-10β					
March '09	CCR7					
Chicken						
June '09	CD80					
June '09	CD83					
June '09	CD86					
March '09	IL-2Rα					
May '09	CXCR4					
Catfish						
√	TCRα					
√	TCRγ					
√ (recloning July '09 into different vector)	TCRβ					
June '09	TCRδ					
√ in bacteria	IgD					
√ in bacteria	IgLλ					
√ in bacteria	IgLσ					
Express in bacteria	B2 micro		X			
√ in bacteria	CD4L1					
	CD8 to be cloned					
Trout						
August '09	TCRα					
July '09	TCRβ					
June '09	TCRγ					
√ @ USGS	Blimp					
√ in bacteria	CD3g/d					
√ in bacteria	CD4					
√ in bacteria	CD4-REL					
√ in bacteria	CD8					
Express in bacteria	CD28	X				
Express in bacteria	CD79A					
@ USGS	CD79B					
Express in bacteria	CD83					
Express in bacteria	CTLA4					
Express in bacteria	IgD					
Express in bacteria	IgT					
Express in bacteria	MHC IA (UBA)	X				
@ USGS	MHC IIB	X				
√ in bacteria	p56LCK					
√ in bacteria	Pax5					

*GenBank # and FASTA files of these genes can be found at www.vetimm.org

All in the **Table 2** will be used to produce mAbs as scheduled below in the central labs at Umass and Cornell or directly in Species labs if not indicated on this schedule

Those indicated by 'Yes' in **Table 1** are slated for mAb production in this round as scheduled in table below; ……..others will be targeted if re-funded

Table 1. CYTOKINE & CHEMOKINE PRODUCTION SCHEDULE

CATFISH	US-VIRN clone's GenBank #	Transformed in expression vector	Expressed in yeast (expected date)	Purified on FPLC (expected date)	Bioactivity affirmed	Will monoclonal antibodies be produced?
IFNγ	DQ124250	1/09	4/09			Yes
Type I IFN	AY847295	1/09	4/09			

CATTLE	US-VIRN clone's GenBank #	Transformed in expression vector	Expressed in yeast (expected date)	Purified on FPLC (expected date)	Bioactivity affirmed	Will monoclonal antibodies be produced?
CCL2	EU276069	√	√			
CCL5	EU276060	√	2/09			
CCL11	EU744565	√	√			
CXCL9	EU276061	√	12/08			
CXCL10	EU276062	√	2/09			
CXCL11	EU276063	√	√	√	YES	
IFN-γ	EU276066	√	√	√	YES	
IFN-α	EU276064	√	√	√	YES	yes
IFN-β	EU276065	√	√			
IL-1β	EU276067	√	√			
IL-2	EU276068	√	12/08			
IL-4	EU276069	√	√	√	YES	
IL-5	EU276070	√	3/09			
IL-6	EU276071	√	√			yes
IL-7	EU276072	√	3/09			
IL-8	EU276073	√	√ (additional 1/09)			
IL-10	EU276074	√	√			
IL-12p35	EU276075	√	2/09			
Il-12p40	EU276076	√	2/09			
IL-13	EU276077	√	√	√	YES	yes
IL-15	EU682380	√	√			
IL-17	EU682381	√	√			yes
IL-18	EU276078	√	√ (additional 1/09)			
IL-23	EU616677	√	1/09			yes
TNF-α	EU276079	√	√ (additional 1/09)			

CHICKEN	US-VIRN clone's GenBank #	Transformed in expression vector	Expressed in yeast (expected date)	Purified on FPLC (expected date)	Bioactivity affirmed	Will monoclonal antibodies be produced?
CCL4	NM0010303 60	√	2/09			
CCL20	NM204438	√	2/09			
IFNγ	AH009942	√	3/09			
IL-1β	Y15006	√	3/09			
IL-2	AF017645		2/09			
IL-4	NM 001007079	√	1/09			yes
IL-10	NM 001004414	√	√	√	YES	Done
IL-12p35	NM213588		4/09			yes
IL-12p40	Ay262752		4/09			
IL-15	NM 204571	√	3/09			yes
IL-16	AJ508678	√	√	√	YES	Done
IL-17	AJ493595		3/09			
IL-17D	EI570583		1/09			yes
IL-18	AJ277865	√	12/08			yes
LITAF	AY765397	√	4/09			
Lymphotactin	AF006742	√	4/09			
MIF	M95776	√	1/09			
TNFSF15 (TL1A)	NM0010245 78	√	2/09			

HORSE	US-VIRN clone's GenBank #	Transformed in expression vector	Expressed in yeast (expected date)	Purified on FPLC (expected date)	Bioactivity affirmed	Will monoclonal antibodies be produced?
CCL2	EU438774	√	1/09			
CCL3	EU438775	√	3/09			
CCL5	EU744564		3/09			
CCL11	In progress		3/09			
CXCL9	EU438776		2/09			
CXCL10	EU438777	√	2/09			
GM-CSF	EU438778	√	√ (additional 1/09)			
IFN-γ	U04050	√	√			
IFN-α1	EU682378	√	√			
IL-1β	EU438767	√	1/09			
IL-2	EU438768	√	√	√	YES	Yes
IL-4	EU438769	√	√	√	YES	Yes
IL-5	U91947		3/09			
IL-6	EU438770	√	√			Yes
IL-10	EU438771		3/09			
IL-12 p35/p40			4/09			
IL-13	EF645663		4/09			
IL-15	EU682379	√	12/08			
IL-17	EU744563-no s.s.	√	1/09			
IL-18	EU438772	√	√			
IL-23	EU438773	√	12/08			
TGF-β	X99438	√	4/09			
TNF-α	EU438779	√	2/09			

SWINE	US-VIRN clone's GenBank #	Transformed in expression vector	Expressed in yeast (expected date)	Purified on FPLC (expected date)	Bioactivity affirmed	Will monoclonal antibodies be produced?
CCL2	EU682382	√	√			Yes
CCL3L1	EU364893	√	3/09			
CCL4	EU364894		1/09			
CCL5	EU44561	√	√			
CXCL9	EU36897	√	2/09			
CXCL10	EU364898	√	12/08	√	YES	Yes
CXCL11	EU682377	√	√	√	YES	Yes
IL-7	EU364895	√	√			
IL-13	EU682383	√	√ (repeat 12/08)	√	YES	Yes
IL-15	NM 214390	√	12/08			
IFN-α	EU364896	√	√			
IFN-β1	EU744562	√	√ (additional 1/09)			Yes
TNF-α	EU682384	√	√			

TROUT	US-VIRN clone's GenBank #	Transformed in expression vector	Expressed in yeast (expected date)	Purified on FPLC (expected date)	Bioactivity affirmed	Will monoclonal antibodies be produced?
IFN1	AM489418	√	12/08			Yes
IFN2	AJ582754	√	12/08			Yes
IFN3	AM235736	√	12/08			Yes
IFNγ	AJ616215	√	12/08			Yes

Table 3. MONOCLONAL ANTIBODY PRODUCTION SCHEDULE

		University of Massachusetts				Cornell
Year	MONTH	Spot 1	Spot 2	Spot 3	Spot 4	Spot 5
07	Jan	(Eq IL-8 – failed)				
08	Jan	(Eq IL-8 – failed)				Eq CD25
08	May	(Eq IL-8 – failed)				Sw IL-4Rα
08	Sept	Catfish TCRγ	Catfish TCRα	Bo TCRγ	Eq CD40	Sw TCRβ
08	Oct	*Ch IL-10*	*Ch IL-16*	Sw CD45	*(Ch IL-10-2nd)*	Eq CD23
08	Nov	(Sw CD45-2ns)	Catfish CD4L1	Catfish IgLδ	Catfish IgLλ	Eq CD28
08	Dec	*Bo IL-13*	*Bo IL-23*	*Sw CXCL10*	*Sw CXCL11*	*Eq IL-4*
09	Jan	*Tr IFN 1*	*Bo IFNα*	*SwIFNβ*	*Sw IL-13*	*Sw CCL2*
09	Feb	Bo TCRδ	*Ch IL-4*	*Ch IL-17*	*Bo IL-17*	*Sw IL-13Rα*
09	Mar	Bo IL-10β	*Tr IFN 2*	Ch IL-2Rα	Catfish β2-micro	Eq FcεRIα
09	April	*Tr IFN-3*	*Ch IL-17D*	Bo CCR7		*Eq IL-2*
09	May	Ch CXCR4	*Tr IFN-4*	*Bo IL-6*	Tr CD3	*Eq IL-6*
09	June	Tr TCRγ	Ch CD80	Ch CD83	Ch CD86	Sw TCRβ
09	July	Tr TCRβ	Catfish TCRδ	Catfish TCRβ		Eq TCRα
09	Aug	Tr TCRα	Tr CD4REL	Bo TCRα**	*Ch IL-18*	Sw TCRα
09	Sept	Catfish Type II IFNγ	Tr CD28		*Ch IL-15*	Eq TCRγ
09	Oct	Catfish TCRβ2	BOVINE*	SWINE*		Eq TCRδ
09	Nov	Catfish IgD	Tr CTLA4	SWINE*	Tr CD83	Eq IgD
09	Dec	Catfish CD8	Bo IL-23R			