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## Abstract

The US Veterinary Immune Reagent Network ([vetimm.org](http://vetimm.org)) was established to address the lack of sufficient immunological reagents specific for ruminants, swine, poultry, equine and aquaculture species. In 2008 major progress has been made to produce sets of reagents for pigs. Recombinant chemokines CXCL10 and CXCL11 were expressed in *Pichia* as and shown to be bioactive; development of monoclonal antibodies (mAb) to them is planned. The cytokines, interleukin-13 (IL-13), IL-15, interferon-alpha (IFN $\alpha$ ) and IFN $\beta$  have been expressed; bioactivity testing is underway. Screening of hybridoma fusions for mAb to T cell receptors, TCR $\alpha\beta$ , and cytokine receptors (IL-4R, IL-13R) have begun using the Cornell Univ. based fusion protein expression system for immunizations and screening ELISAs coupled with flow cytometry of whole cells as a final bioassay. A priority reagent development list and progress update for swine is available at [vetimm.org](http://vetimm.org) as are all bioassay methods and gene sequences. Since many swine cytokine and CD reagents are available commercially the Network website includes a listing of those reagents and is regularly updated. Our goal is to produce reagents that function in ELISA, Luminex assays, ELISPOT and flow cytometric applications as well as in fixed tissue sections. Products developed in this proposal will be openly available to collaborators and be made commercially available using non-exclusive licenses. This project was funded by USDA CSREES proposal 2005-01812 and ARS.

## 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. ([www.vetimm.org](http://www.vetimm.org))  
**Swine Plans:** 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. Butler and Muyldermans (see their poster).  
**Expected Applications:** These reagents will be used to: (1) evaluate changes during disease and following vaccination and (2) give scientists the ability to manipulate these cell populations to evaluate their roles in protective immunity as well as in immunopathology.

## Swine T Cell Receptors (TCRs)

### Example of strategy for expression and mAb production

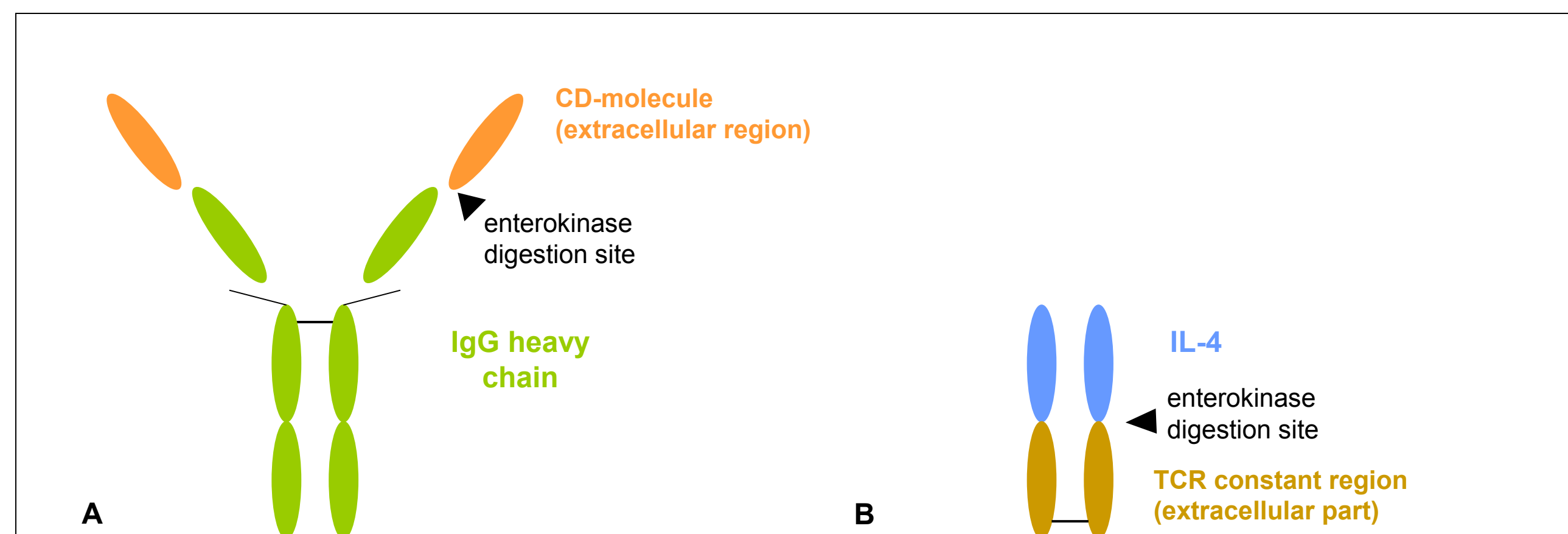
- Bioinformatics (BARC).** The TCRs consist of an alpha and beta chain with variable and constant regions, hinge, transmembrane region and cytosolic tail. For our expression and hybridoma production we are targeting the constant region, the hinge and the area before the transmembrane region. The hinge involves a cysteine disulfide link. The strategy is to produce mAb only to either the alpha or the beta chain constant region, and not the conformational complex (which would require difficult expression strategies). Originally a clone was requested from Salmuller and Uenishi. Fortunately sequence information for swine TCR alpha/beta molecules was available; there were some sequence differences probably due to allelic differences. Primers were designed in collaboration with Dr. D Zarlenga at BARC.
  - cDNA preparation (BARC):** cDNA was produced from pooled immune cDNAs generated from control and infected mini-pigs.
  - PCR product production (UMass):** PCR product produced, cloned and sequence validated.
  - Mammalian expression (Cornell):** Reamplification with restriction sites, insertion into equine IgG1 heavy chain expression vector, stable transfection and clone selection; followed by protein production and purification.
- Final Swine TCR sequence after reamplification at Cornell:**
- Swine TCR alpha**
- ```

1  attcagaacc ctgaccocgg cgtgtaccag ctgaaagggc ccaaatctaa caacatcagt
61  gtatggcctat acactgatgt tcaaatgaa actcggagcc cggatgttc
121  agcctgagca ggaactgtgtt caactcaaac agaactgtgc tagacatggg gcccgggtt
181  tccagagaga acggctgat agctggagg aagagcaaac atttgaatg tcaagcaacc
241  tccagagagg aattctatcc taactcagga atttccggg atgcaagtt ggttagagaaa
301  agctttagaaa cgaatgtgga cctcaacccc caaacctgtt caatgatgg gctccgcatc
361  atctctctga aatgggttgg gtttaacctg ctcattgacac tgggtctga gtcacactga

```
- Swine TCR beta**
- ```

1  gaggcaactgc agcaggtggg accacccaaag gtggccctgt ttagaacatc ggaagcggag
61  atctcccaga cccagagagc cacactcgtg tgcctggcca cagcttctta cccagaccac
121  gtggactgga cctgggggtt gaaaggaaq caggtccaga gggggttag caagacact
181  cagcctatca cggaggagcc cagcccaact gaactcaagct actgtctgag cagcgggttc
241  aggttcacgc cggctctctg gcaacacccc cgaacacact tccctgtgca agtccagttc
301  tagggctgca cagactggag tcaaacctgg caaacctgat caccagagaa
361  atcagtgagg agcctggggg aaagcagaa tctgggtctc cctctggctc ctatcagaaa
421  ggggtctctg ctcccaacct cctctatgag atcctgtgtg gaaagccac cctgacactc
481  gtgctctctg ggcctctgtt actgatggcc acgttaagaa aaaaagatc ctgagaccag

```
- The purple is the coding region for the constant region that will be inserted into the mammalian expression vector. The blue box is the cysteine for the disulfide link at the hinge. The green is the start of the transmembrane region. The green boxes are nucleotide differences from the Japanese clones. The black is the cytosolic area. Cornell forward and reverse primer sites are indicated by italics.
- Hybridoma production (UMass).** Planned.
  - Screening (UMass and BARC):** The initial screen will be run at UMass against the recombinant protein. The functional test will be at BARC against pig T cells by flow cytometry.



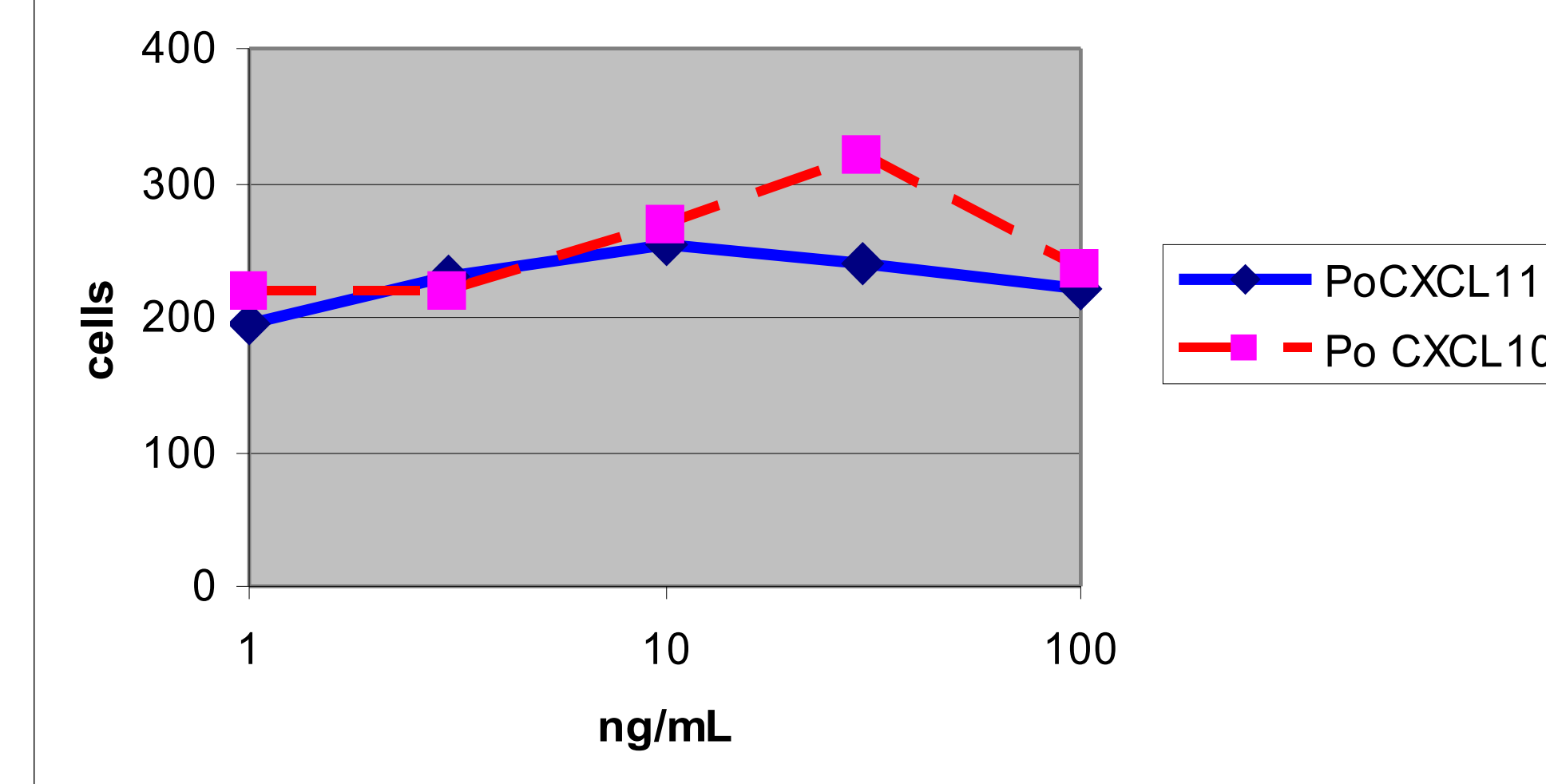
**Figure 1:** Two expression systems are used to generate proteins for six species for the US Veterinary Immune Reagent Network. A) An IgG fusion protein system to express N-terminal proteins (e.g. extracellular regions of CD-molecules or cytokine receptors), and B) an IL-4 fusion protein system that can be used for expression of C-terminal proteins (e.g. TCR or immunoglobulin constant regions).

## Bioassay of expressed Cytokines and Chemokines

### Strategy for Cytokine and Chemokine Bioactivity Assay:

- Screen for general bioactivity on pig, human or mouse cell line, e.g. proliferation of TF-1 cells
  - Test for stimulation of expression of proteins that can be screened using cell lines, e.g. stimulation of PBMC to express IL-6 that can be screened on B9 cells
  - Test for specific bioactivity using relevant pig cells, e.g., chemotaxis of specific receptor + cells, e.g., CXCL10 (Fig.2)
- Assays listed on website ([www.vetimm.org](http://www.vetimm.org))

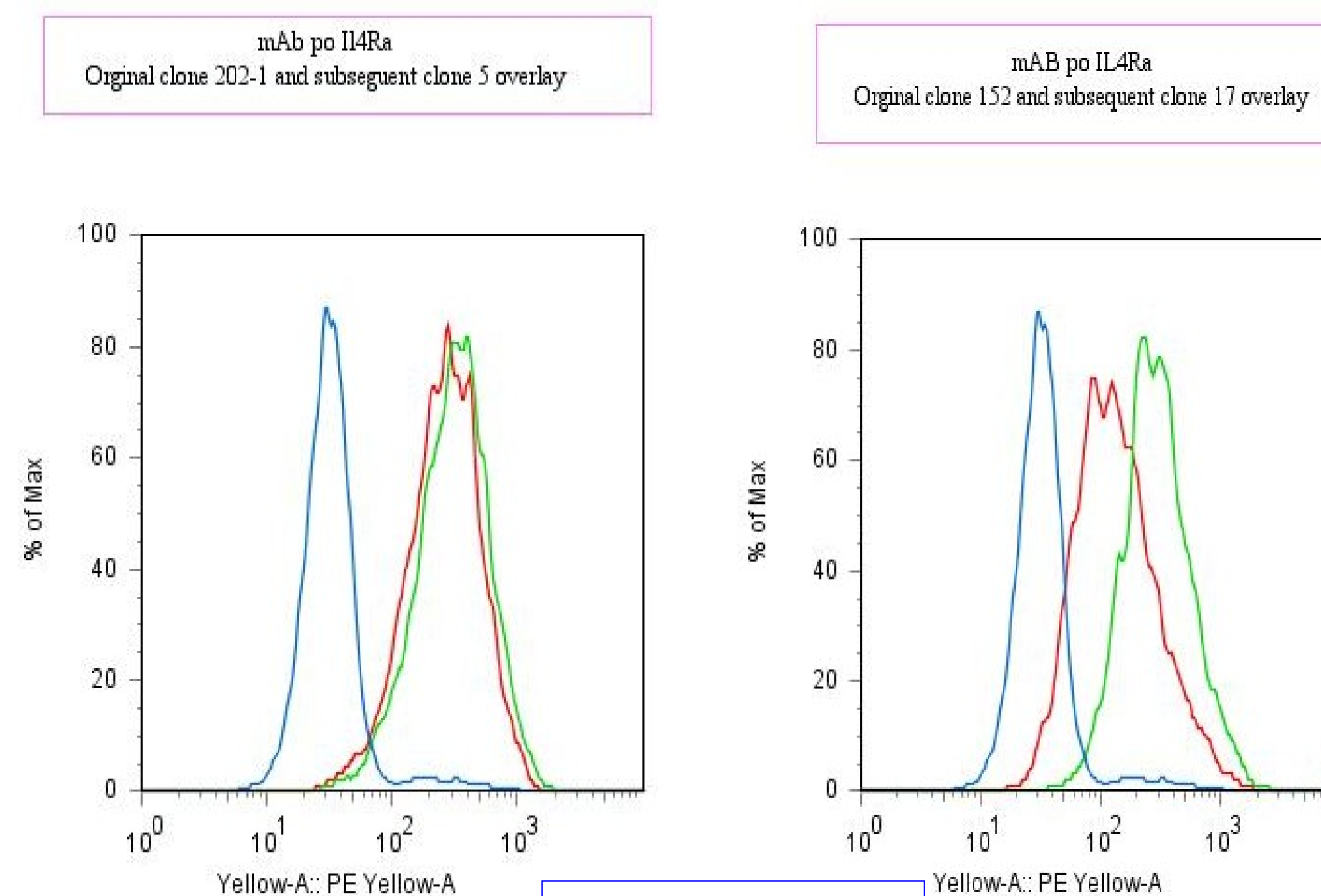
**Figure 2** Bioactivity of poCXCL10 and poCXCL11 [Chemotaxis of pig PBMC after 3d PHA; 1-2d IL-2]



## Table 2. Progress for porcine cell surface molecules

Antigen	Location	Results of Expression, Hybridoma fusion
poTCR $\alpha$ (IgG fp)	Cornell	poor fusion
poTCR $\alpha$ (IL-4 fp)	Cornell	gene is being re-cloned in IL-4 expression vector
poTCR $\beta$ (IgG fp)	Cornell	poor T cell results FCM; gene is being re-cloned in IL-4 expression vector
poIL-4R $\alpha$ (IgG fp)	Cornell	good fusion, mAbs identified using IPEC-J2 cells (Fig.3)
IL-13R $\alpha$ 1 (IgG fp)	Cornell	Fusion 1/09
CD45RO peptide	U Mass	Fusion 12/08
CXCR3	in process	

## Figure 3. Screen of anti-poIL-4R mAb on IPEC-J2 cells



**Fig.3 Legend:**  
 Background Ig  
 Original clone mAb supe  
 Expanded clone mAb supe

## Table 2. Progress for porcine Cytokines and Chemokines

Recombinant Protein	Current Results	Monoclonal Antibody	Location fusion
CXCL10	+ bioassay (Fig.2) chemotaxis	Fusion 12/08	U Mass
CXCL11	+ bioassay (Fig.2) chemotaxis	Fusion 12/08	U Mass
CCL2	ready for bioassay	Fusion 1/09	Cornell
IL-13	Recloned; expressed; weak bioactivity with B9 or TF-1 cells; retest bioassay (W Golde, Plum Island, NY)	Fusion 1/09	U Mass
IL-15	purification in process		
IFN- $\alpha$	purification problems; bioassay MBDK cells; alternate assay = swine test for viral inhibition	No mAb needed	
IFN- $\beta$ 1	purification problems; bioassay MBDK cells; alternate assay = swine test for viral inhibition	Fusion 1/09	U Mass
CCL3L1	Ready for yeast expression		
CCL4	In process		
CCL5	Expressed, not yet purified		
CXCL9	Ready for yeast expression		
TNF- $\alpha$	Ready for yeast expression		
IL-17	not yet on list; may do		
IL-7	Expressed and purified; ready for bioassay		

## Table 3. Update on Expression and Purification of Swine Cytokines and Chemokines

Cytokine/Chemokine	CODING SEQUENCE		PROGRESS FOR EXPRESSION OF MATURE PROTEIN			Purified Protein		
	US-VIRN GenBank #	FASTA file on website	Clone # without s.s.	Stage	Transformed in expression vector	Expressed in yeast	Protein purified	Bioactivity affirmed
CCL2	EU682382	✓	DT-616	Sent to KF	✓	✓	YES	In progress
CCL3L1	EU364893	✓	DT-639	Sent to KF	✓			
CCL4	EU364894	✓		Sent to KF to finish				
CCL5	EU44561 – no s.s.	✓	DT-515	Sent to KF	✓	✓		
CXCL9	EU36897	✓	DT421	Sent to KF	✓			
CXCL10	EU364898	✓	TH-249	Sent to KF	✓	✓	YES	YES
CXCL11	EU682377	✓	DT-432	Sent to KF	✓	✓	YES	YES
IL-7	EU364895	✓	DT-413	Sent to KF	✓	✓	In progress	
IL-13	EU682383	✓	TH-253	Sent to KF	✓	✓	YES	In progress
IL-15	NM 214390	✓	DT-228	Sent to KF	✓	✓	In progress	
IFN- $\alpha$	EU364896	✓	DT-440	Sent to KF	✓	✓	In progress	
TNF- $\alpha$	EU682384	✓	DT-624	Sent to KF	✓	✓		

## Current and Future Plans

### Develop mAb to swine IgG isotypes

Good expression of chimeric camelid-swine IgG constructs for each swine IgG isotype, developed with S. Muyldermans, Belgium on separate NPB grant with J Butler, U Iowa. Mice immunized with chimeric camelid-swine IgG constructs but poor results to date.

### Develop more sensitive assays

Multiplex bead (Luminex) assay grant funded by NPB, to Jane Christopher-Hennings SDSU and others, for developing bead assay for 8-10 cytokines using currently available anti-cytokine mAb reagents.

### Potential for renewal of US VIRN efforts -

## Expand panel of reagents planned

**Give your feedback please!**

## Acknowledgements

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