Expression of recombinant immunoglobulins and cell surface molecules for six species: Cattle, pig, horse, chicken, catfish and trout.

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The US Veterinary Immune Reagent Network (http://www.umass.edu/vetimm) seeks to develop new tools for molecules of the immune system in veterinary species (cattle, pig, horse, chicken, catfish and trout). Recombinant proteins for immunoglobulins and various cell surface molecules, including T-cell receptor chains, CD molecules and cytokine receptors have been expressed at Cornell University. Two mammalian expression systems are used; (1) an IgG fusion protein and (2) an IL-4 fusion protein system. All proteins are expressed in Chinese Hamster Ovary (CHO) cells. Purified recombinant proteins are then submitted to the University of Massachusetts at Amherst for monoclonal antibody production. The first recombinant proteins that have been expressed with this system for the US Veterinary Immune Reagent Network are T-cell receptor constant region domains. These include the bovine TCRγ and TCRδ proteins and the catfish TCRα and TCRβ constant regions. TCR genes of the horse, pig and trout are being processed. Other molecules that have been expressed with the fusion protein system include equine CD40 and the FcεRI γ-chain.

Expression cloning and protein production is ongoing for cattle IL-23R and IL-10R, pig IL-4Ra and IL-13Ra1, equine CD23, CD25, CD28 and the IgD heavy chain, and chicken IL-2Ra and CXCR4

**Introduction**

We previously expressed different recombinant equine cytokines (IL-2, IL-4, IFN-γ, TGF-β) as an equine IgG fusion protein. The cytokines were isolated from the IgG tag by enterokinase digestion (Wagner et al. 2005). Some of the equine recombinant cytokines were already used for successful monoclonal antibody production (Wagner et al. 2006). The same system is now being used to express cell surface molecules and immunoglobulins for six veterinary species as N-terminal proteins (Fig. 1A). To express C-terminal proteins, a fusion protein system based on IL-4 is used (Fig. 1B).

**Material and Methods**

- **cDNA and cloning:**
  cDNA of different genes of interest is provide by Dr. Baldwin’s group (UMASS), the species coordinators of the Network or is cloned at Cornell University (Fig. 2). The genes are amplified and cloned into one of the expression vectors. Transmembrane regions are excluded from expression.

- **Stable transfection:**
  Chinese Hamster Ovary (CHO) cells are transfected with the contructs and stable transfectants are obtained by G418 selection and limiting dilution cloning.

- **Systems to test for expression:**
  Stable transfectants and their supernatants are tested by flow cytometry and ELISA for protein expression (Fig. 3). The fusion protein partners (IgG or IL-4) are used as tags for detection.

**Results**

Proteins expressed for the US Immune Veterinary Reagent Network

The focus for expression during the first 1.5 years of the project was on TCR constant regions for all species and some CD-molecules. Various proteins are already expressed or in the expression pipeline (Table I). Other molecules to be expressed

Several other proteins are on the priority lists and are planned to be expressed during the remaining time of the project. The Network’s website (http://www.umass.edu/vetimm) contains the priority lists of all species.

**References**


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**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).

**Table I: Recombinant proteins expressed at Cornell University.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Send to UMASS</th>
<th>Stable transfectant</th>
<th>Cloned into expression system</th>
<th>Genes received</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>TCRγ</td>
<td>CD40</td>
<td>IL-4Ra IL-13Ra</td>
<td>IL-10R IL-23R</td>
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<tr>
<td>Pig</td>
<td>TCRβ</td>
<td>CD40</td>
<td>IL-4Ra IL-13Ra</td>
<td>IL-2Ra IL-2Rb</td>
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<tr>
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<td>TCRγ</td>
<td>CD25</td>
<td>TCRα</td>
<td>IL-2Ra IL-2Rb</td>
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<tr>
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<td>CD25</td>
<td>TCRα</td>
<td>CXCR4</td>
</tr>
<tr>
<td>Catfish</td>
<td>TCRβ</td>
<td>CD25</td>
<td>TCRα</td>
<td>CXCR4</td>
</tr>
</tbody>
</table>

**Material and Methods - continue**

- **Protein purification:**
  Proteins are purified from serum-free large scale cultures using Protein G (IgG fusion proteins) or anti-IL-4 sepharose (IL-4 fusion proteins). Purified proteins are tested by SDS-PAGE (Fig. 4). Then they are sent to the University of Massachusetts in Amherst for monoclonal antibody production (Fig. 2).

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**Figure 2:** Expression of cell surface molecules - The US Veterinary Immune Reagent Network -

**Figure 3:** Principle of the ELISA to detect IgG fusion proteins. The detection is sensitive and independent of the molecule of interest.

**Figure 4:** SDS-PAGE of a purified IgG fusion protein after enterokinase digestion. A 12% SDS-gel was used and samples were run under non-reduced conditions. The gel was stained with Coomassie blue. The arrows point to A) the recombinant CD-molecule – here the equine FcεRI γ-chain and b) to the remaining IgG constant heavy chain region. M = low molecular weight marker.

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