Developing Antibodies in an Avian Model to Human IL-13 Receptor Alpha 2, A Tumor Associated Antigen

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Abstract
A new approach to cancer treatment is the ability to kill cancer cells while avoiding healthy tissue. A requirement for this strategy is the presence of a molecule found uniquely on the cancer cell and absent on normal cells. Once identified, cells expressing this cancer-associated molecule can be specifically targeted for treatment. A cytokine receptor protein, human interleukin 13 receptor alpha 2 (hIL13Ralpha2), is a cancer-associated protein. Relying on the specific interaction between antibody and antigen, antibodies to hIL13Ralpha2, modified with cytotoxic agents, may seek out and destroy cancer cells over-expressing hIL13Ralpha2 on their surface. To generate an anti-cancer antibody, a significant amount of antigen, the hIL13Ralpha2 protein, was required. We produced and purified a recombinant extracellular portion of hIL13Ralpha2 protein using a prokaryotic expression system. The purified recombinant protein will be used as antigen to generate the anti-cancer (anti-hIL13Ralpha2) antibodies.

Introduction
- Interleukin 13 (IL-13) is an anti-inflammatory cytokine produced by activated T lymphocytes.
- IL-13/IL-4 receptor is found on normal human cells.
- A subclass of IL-4 independent IL-13 receptors has been discovered (Debinski et al 1999).
- IL-13 Receptor Alpha 2, an IL-4 independent receptor, has been found to be overexpressed in many forms of cancer, including Kaposi’s sarcoma, glioblastoma multiforme, pancreatic cancer, and renal cell carcinoma.
- At this time the treatments for cancer include surgery, radiation, and chemotherapy.
- Chemotherapy drugs cannot distinguish between a cancerous cell and a normal cell.
- It has been proposed that IL-13 Receptor Alpha 2 could be used as a target on cancer cells for chemotherapy drugs (Debinski et al 1998; Husain et al 1997).
- One study showed that cytotoxins directed at an IL-13 specific receptor adversely affected glioma (cancerous) cells, but not normal cells (Debinski et al 1998).
- In a second study, IL-13 combined with toxin was highly specific and highly toxic to Kaposi’s sarcoma cells, while having no effect on normal cells (Husain et al 1997).
- Developing a protein to specifically bind to IL-13 Receptor Alpha 2 could allow anticancer agents to be directly targeted to the cancer cells expressing it.

Objectives
- Produce the IL-13 Receptor Alpha 2 protein in a prokaryotic expression system.
- Use the purified protein as an antigen to develop avian antibodies.

Methods
- Insertion and Cloning of Protein in Prokaryotic Expression Vector
- Expression of Protein
- Isolation and Purification of Proteins
- Production of Antibodies

Results

Figure 1. Amplification of PCR product, IL-13 Receptor Alpha 2. Gel shows bands running at ~1000 bp, the length of the gene fragment.

Figure 2. Insertion of the PCR product, IL-13 Receptor Alpha 2 into the prokaryotic expression vector, pQE30-UA-GFP. The linearized vector is running at ~4223 bp. The recombinant linearized plasmid is running at ~5253 bp, the vector size plus ~1000 bp, the insert size.

Figure 3. Testing for directionality, showed samples 1, 2, 3, 5, and 6 running at ~1000 bp, the size of the gene fragment. Sample 4 was no longer used.

Figure 4. Coomassie blue stain following an SDS PAGE gel to verify the protein size in sample 6 before beginning purification. Lane 1 contains the total protein from an E. coli pellet transformed with a negative control expression vector. Lane 2 contains the total protein from an E. coli pellet transformed with an IL-13 Receptor Alpha 2 expression vector, overexpressing the IL-13 Receptor Alpha 2 protein.

Figure 5. Western blot assay for IL-13 Receptor Alpha 2 protein, using an anti-IL-13 Receptor Alpha 2 antibody. Bands are seen in lane 2 (whole extract) and lane 3 (flow-through) at ~81,000 Da. IL-13 Receptor Alpha 2 protein should be seen at ~40,000 Da in lane 2 and lane 4 (elution), but not in lane 3.

Conclusions
- A protein consistent with the size of IL-13 Receptor Alpha 2 was found in transformed E. coli (Figure 4, Lane 2).
- However, following affinity chromatography and Western Blot analysis, the isolated protein had an apparent molecular weight twice the predicted mass, suggesting a possible dimerization of the protein.
- A possible reason for the unexpected purification result could be that the His tag on the protein was not attaching to the nickel in the affinity chromatography.
- The gene sequence was verified by automated DNA sequencing (Elim Biopharmaceuticals, Inc., Hayward, CA).

Future Research
- Continue the attempt to isolate and purify the IL-13 Receptor Alpha 2 protein.
- Inject the purified protein into chickens to produce large amounts of antibodies to the IL-13 Receptor Alpha 2 protein. This would be followed by a purification of the antibodies from the chicken eggs.
- Determine a method of attaching anticancer agents to the avian antibodies.

Literature Cited

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