GRANT PROGRESS REPORT SUMMARY

Grant: 01415: *Development of Anti-IgE Peptide for Treatment of Canine Allergy*

Principal Investigator: Dr. Bruce Hammerberg, DVM PhD

Research Institution: North Carolina State University

Grant Amount: $84,861.00

Start Date: 1/1/2011 **End Date:** 12/31/2012

Progress Report: End-Year 2

Report Due: 12/31/2012 **Report Received:** 12/21/2012

Recommended for Approval: Approved

(Content of this report is not confidential. A grant sponsor's CHF Health Liaison may request the confidential scientific report submitted by the investigator by contacting the CHF office. The below Report to Grant Sponsors from Investigator can be used in communications with your club members.)

Original Project Description:

Treatment of chronic allergic diseases in dogs, often seen as recurring dermatitis, frequently results in less than optimal outcomes. When the disease can be linked to exposure to specific allergens, such house dust mites, desensitization injections can be effective in some individuals when carried out over an extended time; however, most cases are not resolved by desensitization and require a combination of allergen avoidance and anti-inflammatory drugs. The prolonged use of these drugs, such as corticosteroids, can result in severe side effects. These same challenges exist for human allergy suffers, but recently there has been a major breakthrough in the development of a new, safe and effective therapy using a monoclonal antibody that specifically binds and neutralizes human IgE that is responsible for activating inflammation-producing cells. This new product is called Xolair® and it has been used safely by millions of allergy patients for more than 5 years. Our laboratory has developed a monoclonal antibody that specifically binds canine IgE in the same manner as the monoclonal antibody used to develop Xolair®. There are two obstacles remaining in providing the canine equivalent to Xolair® for treatment of allergies in dogs and they are the Objectives of this proposal: 1. Modifying the monoclonal antibody to reduce the dog's natural response to clear this protein; and, 2. Developing cost effective production of the modified antibody. Our Approach is to: 1. Generate a single chain recombinant peptide from the IgE-binding region of our canine IgE-specific monoclonal antibody that is small in size and of limited antigenic potential; and 2. Develop a transgenic plant (eg. tobacco) containing the gene for this recombinant peptide using well established techniques that will allow production of the

therapeutic peptide in kilogram quantities. The expected outcome will be to provide a new, safe and highly effective treatment option for canine allergic diseases that is affordable to use for maintenance therapy.

Grant Objectives:

Objective 1: To create a recombinant, nonanaphylactic, single-chain antibody fragment (scFv) with high affinity for canine IgE from the variable region gene sequences of mAb 5.91 clones.

Objective 2: To generate a plant-derived recombinant, nonanaphylactic, single-chain antibody fragment with high affinity for IgE that can be scaled up for production at kilogram amounts.

Publications:

None at this time.

Report to Grant Sponsor from Investigator:

1) To create a recombinant, non-anaphylactic, single-chain antibody fragment (scFv) with high affinity for canine IgE from the variable region gene sequences of mAb 5.91.

The sequence for the light chain variable region of mAb 5.91 was completed in April, 2011. The sequence for the heavy chain variable region was completed in October, 2011. Linkage of the two sequences and expression of a recombinant scFv of mAb 5.91 with confirmation of high affinity binding to canine IgE was completed in November, 2011.

A Fab fragment was produced from the whole molecule mAb 5.91 and used in flow cytometry assays as a model for the recombinant scFv version of the antibody by May, 2011. Whole blood from allergic dogs was processed and assayed. Results showed that the whole mAb 5.91 molecule reduced the amount of binding of canine IgE to the monocyte cell population from 15% to 7.7%. Moreover, the intact mAb 5.91 was able to bind the free IgE to prevent it from binding cell surface receptors. However, whole molecule mAb 5.91 complexed with canine IgE bound to 13.7% of the lymphocyte cell population possibly reacting with IgG Fc receptors. The Fab fragment of mAb 5.91, pre-incubated with canine IgE, reduced the binding of canine IgE to the monocyte cell population from 15% to 5.6%. This demonstrated that the Fab fragment of mAb 5.91 was even more effective in reducing the binding of IgE to the monocyte cell population than the intact mAb 5.91. There was no evidence of Fab fragment complexed with canine IgE binding to lymphocytes as previously seen with intact mAb 5.91.

These preliminary results indicate that the recombinant scFv form of the mAb 5.91 will be more effective at blocking IgE binding to cell surface receptors as well as decreasing the potential of cross reactivity of the lymphocyte cell population with the IgG Fc receptors than the original mAb 5.91.

2) To generate a plant-derived recombinant, nonanaphylactic, scFv with high affinity for IgE that can be scaled up for production at kilogram amounts. To be completed in the second year. Gene constructs of the newly made 5.91-scFv were designed to target the chloroplast and ER regions of the tobacco leaf cells. Both gene constructs were inserted into a PVX pGR106 amplicon vector and amplified in E.coli. The purified 5.91scFv-pGR106 constructs are being used to transform Agrobacterium tumefaciens strain GV3101. However, problems have been encountered during transformation attempts of Agrobacterium tumefaciens with the purified 5.91scFv-pGR106 constructs. A second round of transformation is being performed at this time.

TEV-B is a transgenic tobacco plant that expresses a mutated P1/HC-Pro suppressor of Post Transcriptional Gene Silencing. It has been shown that this line of tobacco plants produces higher protein yields than wild type varieties of tobacco including Nicotiana benthamiana. TEV-B seeds were planted on May 23rd and TEV-B plants should be ready for infection in July. TEV-B plants were Agroinfected with Agrobacterium tumefaciens GV3101 containing the gene 5.91scFv::chloro gene construct. Total protein was extracted from 2kg of transgenic leaf tissue. Crude extract was clarified from photosynthetic proteins and polyphenols that may interact with downstream applications.
Binding activity of the 5.91scFv in the extract was confirmed on ELISA and was later compared to the activity of 5.91Fab and intact mAb 5.91 in whole blood and canine mast cells flow cytometry assays.