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Bachelor of Science in Animal Science

Detection of Differentially Expressed Genes Involved in Immune Function

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ABSTRACT

The effect of stress on the immune system of mammals has been traced to the fact that glucocorticoids are released. The effect of glucocorticoids on the immune system can be further investigated by studying the effects of these glucocorticoids on genes with specific immune functions. In animals treated with glucocorticoids (ACTH and dexamethasone), cDNA- AFLP was used to identify genes with differential expression. Two of these genes, TCTP and LAMR1, had specific immune functions and will be studied further in future research. The experiment showed that the glucocorticoids did in fact cause differential expression of the genes. The next step is to do an RT- PCR with the two immune- related genes to further understand the degree of differential expression.

INTRODUCTION

Scientists have long been trying to understand all the factors that control gene expression. By examining what controls all of the genes many researchers hope that someday they can control which genes are expressed. This process has many proposed uses perhaps the most intriguing of which is to prevent diseases with a known genetic link. There are a couple of ways to characterize control of gene expression. First, the gene can be turned off so that no product is formed. The other method is to control the concentration of product created. The effect of blood steroid concentrations on genes is unknown but there is evidence to suggest a relationship.

Glucocorticoids are most commonly known as anti- inflammatory drugs but there are many more functions being studied such as immunosuppression. These steroids are already diagnosed for autoimmune diseases but researchers are trying to discover why they inhibit the immune system. Glucocorticoids are also released in response to stress in

animals. When stressed, animals undergo immunosuppression an effect that many think may be related to this physiological response. (Romero 1992)

The exact mechanism by which glucocorticoids suppress immunity is still relatively unknown, but the effect is well documented. (Marx 1995) By examining the effect of glucocorticoids on gene expression, it may be possible to find a gene specifically related to immunity affected by glucocorticoid levels. Pigs treated with dexamethasone and ACTH were screened for differential expression using a process known as cDNA- AFLP (amplified fragment length polymorphism). A number of genes showed differential expression but two (TCTP and LAMR1) were considered functional candidates because of their known immune functions. By cloning these sections and putting them through RT- PCR (reverse transcriptase- polymerase chain reaction), it is even possible to see when the expression of these genes is affected.

METHODS AND MATERIALS

Animals

Twenty- four pigs were chosen from eight litters when they were 8 weeks old. They were separated into two groups and randomly assigned one of three treatments: ACTH (1 IU/kg BW, 10 IU/ml in 0.9% NaCl, Sigma A6306), dexamethasone (16 μg/kg BW, 100 μg/ml in 0.9% NaCl and 5% ethanol, Sigma D1756), or the control (2.5 ml 0.9% NaCl). All treatments were given via an indwelling catheter. Blood was collected four hours after treatment over a one-hour period every fifteen minutes. Two mls of blood was drawn before each 5 ml sample and discarded. (Adcock 2006)

cDNA- AFLP

RNA was isolated from the whole blood then used to create cDNA using para-magnetic beads. One strand at a time was created using reverse transcriptase and collected on the beads. The cDNA was digested with two enzymes and known adapter sequences were added. The cDNA then underwent two PCR reaction in a thermal cycler to amplify the DNA (primers were complementary to the adapter sequences). In the second round of PCR, selective amplification was performed to limit the number of products. (Rairdan 2001)

High Resolution Gel

The product of the final PCR was run on a high-resolution agarose gel. The fragments that showed differential expression were cut out and cloned. They were then re-amplified, purified, and sequenced for identification. Two genes that showed differential expression were chosen as functional candidates based on their documented immune-related function.

RESULTS

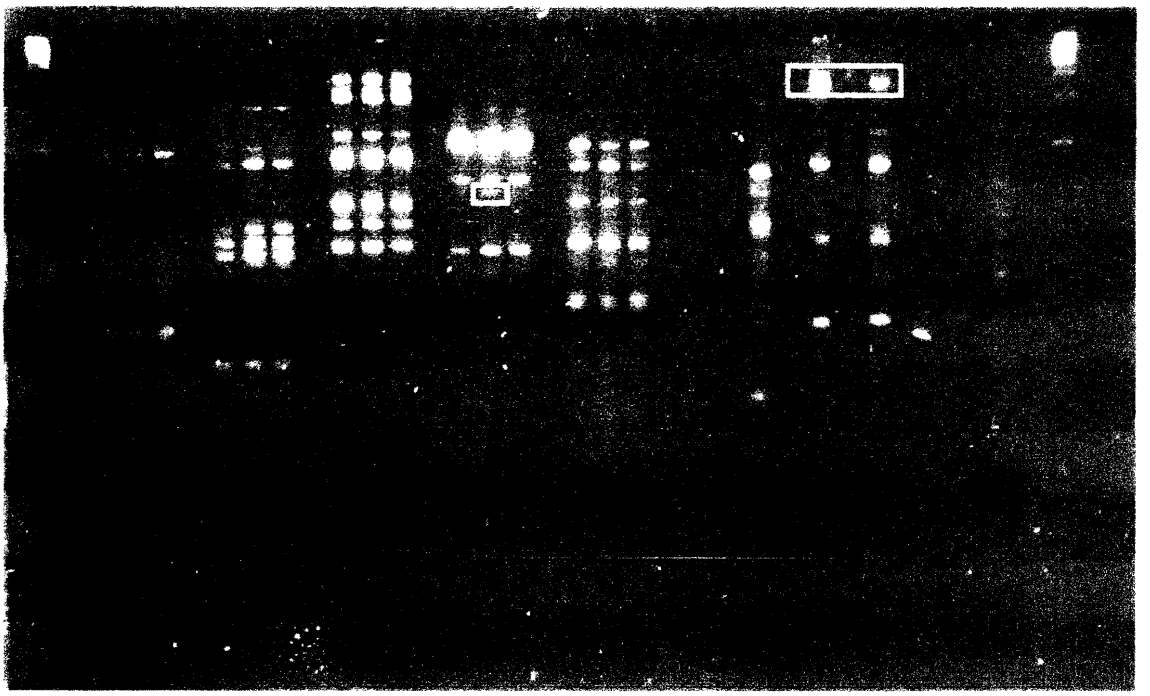

Figure 1. High-resolution agarose gel with genes showing differential selection highlighted.

Sus scrofa mRNA for laminin receptor precursor (LAMR1 gene)
Length=1084
Query 1 GCAGCCTTCCGGGAGCCAAGACTTCTGGTGGTTACTGATCCCAGGGCTGACCACCAGCCT 60 Sbjct 445 ..... 504 Query 61 CTCACAGAGGCCTCTTAT 78 Sbjct 505 522
Sus scrofa translationally controlled tumor protein (TCTP) mRNA
Length=849
Query 1 TATCACCCGTCGTCATAACTGGCTGGCCACTGCTTTCCATCCACAAAACACCAGGACTTA 60 Sbjct 645 ..... 704 Query 61 GACAAGTGGGACTGATGGCATCTTGAGCTCGTTATTTTGACATTGATTTTTCTTTGGAGC 120 Sbjct 705 ..... 764 Query 121 GGAGGCATTGTTTTTGAGG CATGTCATGTAGGTTGTCTAAAAATAAAATGCAT 180 Sbjct 765 824

Table 1. The sequences of TCTP and LAMR1 from DNA cloned in the lab.

The first significant results were seen in the high-resolution gel (Fig 1) in which the bands highlighted show differential expression. Many of these genes had no relationship with the immune system but two, TCTP and LAMR1, showed a clear functional relevance and were examined further. The amount of difference and when in the process it is affected is measurable only with further reaction. Table 1 shows the sequences of these two genes that were sequenced from the cloned DNA.

DISCUSSION

The genes selected for further study were chosen based on their immune function. TCTP or TPT1 is a tumor protein-releasing gene. It has immune function directly related to allergic reactions. It has been found to release histamine (Bommer 2004) and the protein is even found in allergic blister fluids in patients (Amzallag 2004). TCTP is identical to the histamine releasing factor (Rinnerthaler 2006) and therefore plays a role in inflammatory responses of the immune system. This role specifically relates to this experiment because dexamethasone is used to block the production of histamine.

LAMR1 is associated with high white blood cell counts, an immune response, in tumors in patients with myeloid leukemia (Greiner 2006). LAMR1 has a direct relationship with tumors and is found in higher concentrations in active tumors. Laminin the protein that LAMR1 binds is also found in tumors and was first identified in a mouse tumor. (Palm 1983) The primary function of laminin is as a protein in the basement membrane of cells and it plays a role in lymphocyte migration.(Ganapamo 1996) The lymph system plays an important role in immunity in mammals.

The results of this experiment look promising; there was differential expression of genes with known immune functions. The next step is to perform an RT- PCR on the

DNA to look more closely at the differential expression. The initial results of this step are also promising but are not included due to the time restrictions on this experiment. The optimization of the RT- PCR could not be completed in time and the results of that step will be published later.

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