AUSTRALIAN POULTRY CRC

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Executive Summary

Major problems faced by the Australian poultry industry include: reduced productivity due to disease, reduced reliance on antibiotics and how to respond to growing public concerns over welfare issues. The availability of the full chicken genome has provided a wealth of information and has revolutionised the design, scope and implementation of biological studies. In 2004 we initiated this project to develop research strategies that employ a genomics approach as a platform technology for improved poultry health and performance.

This project encompassed three interconnected strategies focused on developing and applying new genome-based technologies and immunological interventions for the development of improved health products:

Strategy 1. Application of functional genomics for discovery of new health products. (Leader Dr Rob Moore). The major objective was to establish (as a CRC-CSIRO co-investment) a state-of-the-art national facility for poultry immunology and genomic research to service the Australian poultry industry. This expertise is used by the current project, as well as other CRC projects, to understand the response of the chicken to pathogens and will allow the analysis of the genetic expression networks that play fundamental roles in the chicken's biological responses to disease, vaccination, diet, and stress. An understanding of these networks will allow the design of optimal intervention strategies to improve immunological responses, disease resistance, and productivity.

Strategy 2. Development of RNA interference for genomic studies and therapeutic applications (Leader Dr Tim Doran). RNA interference (RNAi) is an exciting new technology that has potential for therapeutic applications for control of viral infection and to control expression of genes with important production benefits, in particular genes that determine sex differentiation and genes regulating muscle development.

Strategy 3. Enhancement of immune responses. (Leader Dr Andrew Bean).
A major deficiency in managing disease control involves a closer understanding of the nature of protective immune responses. In order to rationally design therapeutics and vaccines for a particular disease it is critical to first understand the nature of the protective immune response and then replicate that response during a control strategy. Newly identified candidates can then be assessed for their ability to act as immune enhancers, vaccine adjuvants or therapeutics. These new

insights into the early processes of immune responses will offer improved vaccination and control strategies for a wide range of pathogens.

Major Outcomes:

This project has generated commercially valuable IP two areas, both of which have the potential to significantly improve the competitiveness of the poultry industry. Three International patents have been filed. One technology involves the development sophisticated RNAi constructs that can alter the phenotypic traits of poultry. This development may lead to the ability to control the sex of chickens (by silencing particular sex-determination genes) or to enhance muscle growth in chickens (by modulation of developmental genes). Both of these uses have major commercial potential and address significant welfare issues. The second technology involves the discovery of a new immuno-modulator, interferon lambda. This technology is expected to have applications as a vaccine adjuvant and therapeutic agent for viral diseases.

Other outcomes include:

- The establishment of a National Facility for Immuno-genomics
- Establishment of disease models for immune profiling of CAV and IBV
- Animal trials establish utility of novel adjuvants and therapeutics *(IL-6 and IFNλ)*
- RNAi technology has been developed and validated for in vitro and in ovo use as a gene knock-down tool to study the function of genes identified by genomic studies
- Developed proof-of-concept for RNAi gene silencing to control sex determination
- Development of a bacterial delivery vector for the administration of new therapeutics
- Major input and resource for new gut health project in Program 1 (05-09)
- Major input into formation of a new project for the development of a novel RNAi based MDV vaccine (04-14)
- Successful training of three Post Docs and 3 PhD students

Commercial potential:

We envisage future commercial applications in two areas that the CRC and CSIRO has a strong competitive position in:

1. RNAi Technology: This technology potentially provides a mechanism to significantly improve the efficiency of poultry production. For the egg laying industry the ability to hatch all chicks as females would result in a more efficient and welfare-friendly system as it would eliminate the need for the wasteful mass disposal of male chicks.

2. Interferon Technology: Interferon lambda is a newly identified natural immuno-enhancer that has health benefits such as anti-viral and vaccine adjuvant activity. It has the potential for in ovo use in the poultry industry.

Introduction

Background

A major problem faced by Australian poultry industries is reduced productivity due to disease, which is compounded by the fact that newly hatched chicks are highly susceptible to infection during the first two weeks of life. Over the past several decades the two main mechanisms used to control disease have been the use of vaccines and antibiotics. Vaccines are intended to offer long-term immunity and protection against a particular pathogen following immunisation. In contrast, antimicrobials, such as in-feed antibiotics and chemicals, provide broad-spectrum protection and their use is intended to prevent bacterial disease. In addition to controlling disease, antibiotics also have growth promoting activity, making them a more attractive product. Unfortunately, the extensive use of antibiotics and chemicals over a long period of time has resulted in the emergence of pathogens that have become resistant to such treatments. Furthermore, growing public concern over these issues has resulted in a reduced reliance on antibiotics, resulting in an urgent need for alternative methods for disease control.

Vaccination strategies using live vaccines have in the past provided effective protection from disease, however, there are concerns over their ability to adequately protect against emerging hyper-virulent strains of pathogens. For several diseases of commercial importance there is a need for alternative vaccines. Killed and recombinant subunit vaccines rarely provide adequate levels of long term protection and often require the use of adjuvants to enhance their activity. At the time of initiation of this project, there is a lack of suitable, cost effective adjuvants for use in both the broiler and egg industries.

The availability of detailed genomic information about an organism has revolutionised the design, scope and implementation of biological studies. Since the chicken is the first livestock species for which the entire genome sequence is available, there is now an exciting opportunity to develop research strategies that will employ a genomics approach as a platform technology for improved poultry health and performance. CSIRO AAHL has recently made a large capital investment in microarray technology. As part of this project, we propose to establish a state-of-the-art national facility for poultry immunology and genomic research that will service the Australian poultry industry. Microarray experiments will identify particular host genes that are up or down regulated during infection. Manipulation of these identified genes will allow the development of methods to enhance disease resistance. Similar types of genomic analysis can be used to identify novel therapeutics or antimicrobial peptides such as defensins and new cytokines or to develop better diagnostic tools. This facility can also be used by other projects within the CRC to study the expression of pathogen genes and for the identification of potential new vaccine antigens.

RNA interference (RNAi) is an exciting new technology that involves the use of small interfering double stranded RNA (siRNA). RNAi is a protective innate response observed in a wide variety of organisms, and is a feature of all eukaryotes. At the cellular level, the detection of a double stranded RNA molecule triggers the specific degradation of the mRNA of an exact sequence to an expressed gene, leading to loss of phenotype. This technology has been developed as the method of choice to produce gene knockouts for high throughput functional genomic studies in a number of species, opening the way to utilize this for studies in the chicken. As well as genomic studies, RNAi has potential for therapeutic applications. For example, siRNA molecules against essential viral genes have been shown to reduce viral loads in cell culture. Gene specific therapeutics for cancer and auto-immune diseases are also being investigated and show promise in in vitro systems. A major obstacle to overcome in the development of RNAi therapeutics is efficient delivery of siRNA's into target cells in vivo. This has been dramatically advanced by CSIRO's discovery that small hairpin loop RNAs (shRNAs), transcribed from DNA elicit RNAi in vertebrate animal cells. This now enables DNA delivery systems such as plasmids, bacterial and viral vectors to be developed for targeted delivery of shRNAs to animals for therapeutic RNAi use.

The potential to use RNAi to control expression of genes with important production benefits will also be investigated, in particular genes that determine sex differentiation and genes negatively regulating muscle development.

Cytokines are proteins that control immune responses following infection or vaccination. And represent excellent, naturally occurring therapeutics. Cytokine therapy has been successfully used in humans for the treatment of immunodeficiencies, in particular, patients suffering from an impaired immune system as a result of cancer treatment have had their immune responses restored following administration of particular types of cytokines called colony stimulating factors. This enabled them to combat pathogenic organisms that would have otherwise overwhelmed them. This is a situation analogous to the immunodeficient nature of newly hatched chickens or chickens infected with immunosuppressive viruses such as CAV, MDV and IBDV. Cytokines such as IL-6, IL-15 and IL-18 have been shown in mammals to play crucial roles in innate immunity and these same cytokines have recently been discovered in the chicken and are available to study. There are many different types of cytokines that perform different functions. This project will undertake studies assessing the biological function of different cytokines and assess their ability to manipulate the the innate and adaptive immune systems and enhance vaccine efficacy. The capacity of cytokines as natural alternatives to antibiotics will also be studied.

This Project encompasses 2 major themes:

1. Establish a national facility for immunology and genomics-based technology in poultry
2. Application of new technologies for the development of novel health products and improved vaccines.

Extensive information about the chicken genome and its expressed genes is now becoming available, so it is ripe for study at the functional genomics level. This project established a National Facility for Poultry Immunology and Genomics to facilitate the widespread availability of genomic and immunological tools to the poultry research community in Australia. The work is far ranging and requires the development of several new and emerging technologies.

Industry Outcomes:

- Development, application and commercialisation of improved health products and diagnostic tools
- Adoption of new vaccine strategies and integration with other control strategies
- Increased production efficiency of the Australian poultry industry
- Significant generation of IP

Research Strategy:

This proposal contains three interconnected strategies that are focused on developing and applying new genome-based technologies and immunological interventions for the development of improved health products.

Strategy 1. Application of functional genomics for discovery of new health products.

(Leader Dr Rob Moore)

Our major objective is to establish a National Facility for Poultry Immuno-Genomics. This expertise will be used to understand the response of the chicken to various challenges, initially focused on immune responses to pathogen challenge, but expanding into other areas in the future. The principal resource required for these studies will be the development and refinement of suitable microarrays. The microarrays will consist of collections (libraries) of several thousand, appropriate, defined, expression sequence tag (EST) clones. Such microarrays will allow the analysis of the genetic expression networks that play fundamental roles in the chicken's biological responses to disease, vaccination, diet, and stress. An understanding of these networks will allow the design of optimal intervention strategies to improve immunological responses, disease resistance, and productivity.

Outcomes

- The establishment of a National Facility for Immuno-Genomics, will provide a state-of-the-art resource to a range of projects and collaborators within the CRC.
- The planned long-term outcome of the project is the identification and development of novel, natural therapeutics, such as antimicrobial proteins and cytokines.
- It is anticipated that the functional genomic analysis will also provide new analytical tools to monitor poultry health and welfare status.
- The study of functional genomics will provide fundamental insights into poultry biology.

Strategy 2. Development of RNA interference for genomic studies and therapeutic applications (Leader Dr Tim Doran)

RNA interference (RNAi) has already been developed as the method of choice to produce gene knockouts for high throughput functional genomic studies in a number of species. As well as functional genomic studies, RNAi has potential for therapeutic applications for control of viral infection and to control expression of genes with important production benefits, in particular genes that determine sex differentiation and genes regulating muscle development.

Outcomes

- RNAi developed for routine in vitro and in ovo use as a gene knock-down tool to study the function of genes identified by genomic studies, including those responsible for sex-determination and muscle development (see Strategy 1).
- RNAi developed as an anti-viral agent (e.g. CAV)
- Generation of IP and potential commercial products identified via proof-of-concept.

Strategy 3. Enhancement of immune responses. (Leader Dr Andrew Bean).

A major deficiency in managing disease control involves a closer understanding of the nature of protective immune responses. In order to rationally design therapeutics and vaccines for a particular disease it is critical to first understand the nature of the protective immune response and then replicate that response during a control strategy. This involves studying the innate immune response and the cytokines produced during infection by the pathogen in question as well as the immune cell populations affected. With the recent publication of the full chicken genome, we now have access a large number of avian immune response genes, and with the recent development of Real Time qPCR, we can now accurately measure the profile of these genes during the course of an infection. As we build on our understanding of how cytokines control the immune system, we will gain further insight on how to optimise immune responses to vaccination and how to transform our disease control strategies.

In particular, we will study innate receptors such as Toll Like Receptors (TLR), which direct the immune response and determine which cytokines are produced. Our approaches will make use of this protective immune control point to enhance protection. Cytokines such as IL-6 and -18 have been shown in mammals to play crucial roles in innate immunity and these cytokines have recently been discovered in the chicken and are available to study. The technologies used will rationally determine which combinations of immune molecules are likely candidates as therapeutics. Genomic strategies will also allow us to discover new immune molecules and measure their biological activities. The best candidates can then be assessed for their ability to act as vaccine adjuvants or therapeutics. These new insights into the early processes of immune responses will offer improved vaccination strategies for a wide range of pathogens.

Outcomes

- Improved understanding of innate immune responses during infection (e.g. CRD and CAV)
- Identification of novel TLR's, and innate cytokines
- Development of novel approaches for improving the health of poultry:
    - Novel therapeutics for reduced reliance on in-feed antibiotics and chemicals
    - Improved adjuvants for new vaccines against hyper-virulent pathogens
- Generation of IP and potential commercial products identified via proof-of-concept.