

ABSTRACTS
2003 Poultry Science Association
Annual Meeting
July 6–9, 2003

* Author Presenting Paper

Ancillary Scientists Symposium

1 Proteomics in the chicken: tools for understanding immune responses to avian diseases. S. C. Burgess*, *Mississippi State University, Mississippi State, MS 39762-6100.*

The entire chicken genome sequence will be available by the time this review is in press. Chickens will be the first production animal species to enter the “post-genomic era”. This fundamental structural genomics achievement allows, for the first time, complete functional genomics approaches for understanding the molecular basis of chicken normo- and patho- physiology. The functional genomics paradigm, which contrasts with classical functional genetic investigations of one (or few) genes in isolation, is the systematic holistic genetic analyses of biological systems in defined contexts. Context-dependent gene interactions are the fundamental mechanics of all life. Functional genomics uses high throughput large-scale experimental methods combined with statistical and computational analyses. Chicken expressed-sequence-tag projects have already allowed the creation of cDNA microarrays for large-scale context-dependant mRNA analysis (“transcriptomics”). However, proteins are

the functional units of almost all biological processes and protein expression very often bears no correlation to mRNA expression. Proteomics, a discipline within functional genomics, is the context-defined analysis of complete complements of proteins. Proteomics bridges the “sequence to phenotype gap”; it complements structural and other functional genomics approaches. Proteomics requires high capital investment but has ubiquitous biological applications. Although currently the fastest growing human biomedical discipline, new paradigms may need to be established for production animal proteomics research. The prospective promise, and potential pitfalls, of using proteomics approaches to improve poultry pathogen control will be specifically highlighted. The first stage of our recently-established proteomics program is global protein profiling to identify differentially expressed proteins in the context of the commercially important pathogens. Our trials and tribulations in establishing our proteomics program, as well some of our initial data to understand chicken immune system function, will be discussed.

Key Words: Functional genomics, Proteomics, Immune response, Genome

Immune System Functions and Mechanisms

2 Our current understanding of humoral immunity of poultry. T. R. Scott*, *Clemson University.*

With the inception of the modern era of avian immunology in the mid-1950s following the discovery of the role and importance of the bursa of Fabricius (BF), steady and persistent research has been conducted by scientists to this day. Findings that have advanced our knowledge of humoral immunity in all species have included such things as the distinct role of the B cell in antibody production, the explanation of antibody diversity by gene conversion, the identification of a bursa secretory dendritic cell (BSDC) and DC of the spleen, characterization of cytokine factors produced by immune and stromal cells, and mapping of MHC class II genes. Numerous experiments have been conducted over the years to evaluate the humoral immune responses of poultry through antibody titer determinations and quantitation of immunoglobulin (Ig) concentrations in the body humors. These antibody studies have also included the genetics of humoral immunity regarding both quantity and persistence of responses as influenced by multiple genes and MHC haplotypes. In

the past decade or more, the emergence of many monoclonal antibody reagents employed to identify cell surface markers on B cells and accessory cells has allowed for the discrimination of humoral immune functions of cells by immunohistochemistry, flow cytometry and other techniques. Continued work with avian immunity has led to more advances in the identification, characterization and gene sequencing of cytokines important in this area. Additional work is required to identify and sequence the genes for many more cytokines that have direct effects on B cells for growth, differentiation and Ig class switching. A brief history of the research published on humoral immunity of poultry will be presented followed by an examination of some of the important research findings reported in recent years.

Key Words: Humoral immunity, B cells, Antibodies, Genetic influences, Cytokines

3 Avian cell-mediated immunity. G. F. Erf*, *University of Arkansas, Fayetteville, AR 72701.*

In avian species adaptive immunity involves both humoral and cell-mediated immune (CMI or Th1) responses. While humoral or antibody-mediated immune responses are particularly effective against extracellular microorganisms, CMI responses are especially important for destroying intracellular bacteria, eliminating viral infections and destroying tumor cells. CMI responses, like most humoral immune responses, are tightly regulated and require "help" from T helper (Th) cells, specifically the type 1 Th cells (Th1, hence the name Th1 responses). Th1 cells are characterized by their production of cytokines such as interferon-gamma (IFN- γ) and interleukin-2 that drive CMI responses. The functional effectors of CMI responses are various immune cells, such as cytotoxic T cells (CTLs), macrophages, and natural killer (NK) cells. CTLs kill virus-infected cells and neoplastic cells. Activation of CTLs requires specific recognition of antigen by the CTLs' antigen-specific T cell receptors and a second signal from cytokines produced by Th1 cells. Macrophages, which are cells of innate immunity, will phagocytose the antigen and kill it intracellularly. However, specialized intracellular microorganisms (e.g., bacteria, parasites), once engulfed, can survive within the macrophage unless the macrophage becomes highly activated. Th1 cells that have become activated in response to the intracellular microorganism will provide cytokines (e.g., IFN- γ) that will help macrophages reach the level of activation necessary to effectively eliminate intracellular microorganisms. NK cells, also known as large granular lymphocytes, are generally non-specific but can recognize and kill neoplastic and virus-infected cells. NK cell activity can also be greatly enhanced by Th1-mediated cooperation between adaptive and innate immunity. In the past 10 years substantial progress has been made in defining the role and regulation of avian CMI responses and in addressing strategies that strengthen this arm of adaptive immunity to optimize defense and protection against neoplastic diseases and non-neoplastic diseases caused by intracellular pathogens.

Key Words: Cellular immunity, T helper cells, Cytokines, Cell signaling

Genes Impacting Immune Responses

5 Distinctive polymorphism of chicken B-FI (class I MHC) molecules. Sandra Ewald* and Emily Livant, *Auburn University, Auburn, AL U.S.A.*

The major histocompatibility complex (MHC) in chickens influences disease resistance, but the mechanism is not understood. Candidates for disease resistance genes within the MHC include class I genes. In leghorn lines, the MHC contains two closely-linked class I loci, BFI and BFIV, although in some haplotypes the BFI locus reportedly is disrupted. Previously, we determined nucleotide sequences of well expressed class I (B-F) genes from unique MHC haplotypes of broiler chicken lines. From some haplotypes, we obtained two B-F cDNA sequences and from others only one. More recently, we identified seven new B-F a1a2-coding sequences from less well-expressed loci, by amplification of genomic DNA from unique broiler haplotypes. Phylogenetic analysis of chicken MHC class I a1a2-coding sequences resolved two clusters (Groups A and B), which appear to correspond to BFIV and BFI locus, respectively. Compared with B-FIV locus, B-FI alleles were less polymorphic overall, but nevertheless demonstrated evidence of diversifying selection. The most striking feature of B-FI alleles is a conserved, locus-specific motif in the alpha helix of the a1 domain, a region that is highly variable in B-FIV alleles. This distinctive pattern of allelic polymorphism resembles the HLA-C class I locus in the human MHC. Whereas class I alleles encoded by the HLA-A and -B loci are highly polymorphic, HLA-C alleles are characterized by much less polymorphism, with conservation and locus specificity of amino acid residues in the alpha helix of the a1 domain of the protein. This conservation probably relates to two unusual features of HLA-C molecules: (1) their restricted repertoire of bound peptides; and (2) their interaction with members of the killer immunoglobulin-like receptors (KIR) on natural killer (NK) cells that are specific for recognition of HLA-C molecules and function to regulate activation of NK cells. Whereas HLA-C molecules may be a dominant ligand for NK cell regulation, HLA-A and -B molecules are more important in presenting antigen to cytotoxic T lymphocytes (CTL). We hypothesize that chicken

4 Participation of the intestinal epithelium and mast cells in local mucosal immune responses in commercial poultry. D. J. Caldwell*¹, H. D. Danforth², B. C. Morris³, K. A. Ameiss¹, and A. P. McElroy³, ¹Texas A&M University, College Station, TX, ²USDA/ARS/LPSI/PBEL, Beltsville, MD, ³Virginia Tech, Blacksburg, VA.

The intestinal mucosa of commercial poultry is continually subjected to invasion or colonization by a wide-array of potentially hostile enteric pathogens. While recent investigations have focused on lymphocyte involvement in local immune responses in the intestine, lymphocyte-mediated immunity alone will not explain the barrier nature of mucosal membranes associated with rejection of many enteric pathogens upon secondary homologous challenge. Our laboratories have focused on non-traditional elements of mucosal immunity in poultry to better understand host-pathogen interactions in the intestine. Following classical and novel immunization procedures, we have identified an antigen-specific mechanism of immediate responsiveness of the mucosal epithelium characterized by epithelial chloride secretion. This mechanism, characteristic of intestinal anaphylaxis, appears to be mediated by local immune elements. Similar mechanisms described in mammals contribute to the barrier nature of mucosal membranes during pathogen challenge. Further, to identify cells participating in these and similar responses, additional studies have described a role for mast cells in acute phase responses in the intestine of chickens experimentally challenged with *Eimeria*. To a more practical end, other experiments in our laboratories have characterized drinking water administration of a bovine serum albumin (BSA) for elicitation of local and systemic antibody responses. These experiments have shown *ad libitum* drinking water administration of BSA to be as effective as intraperitoneal administration of BSA and potentially describe a novel approach to immunization of commercial poultry with purified or recombinant protein vaccines. Taken together, these investigations support continued research on host-pathogen interactions within the intestine of commercial poultry to better understand and control enteric pathogens through vaccination or immunomodulation.

Key Words: Mucosal immunity, Intestinal epithelium, Mast cells, Antibody

B-FI molecules may be specialized to serve similar functions as HLA-C molecules.

Key Words: Major histocompatibility complex, Broiler, Natural killer

6 The genes of innate immunity on chicken chromosome 16. M. M. Miller*, *Beckman Research Institute, City of Hope National Medical Center.*

Increasingly the major histocompatibility complex (MHC) is being recognized for its role in innate immunity. Diverse interactions occur between MHC class I molecules and receptors present on natural killer (NK) cells. These interactions result variously in activation or inhibition of NK cells in activities important in recognizing disease and controlling immune responses. In mammals, the NK cell receptors are products of large, polymorphic gene clusters located away from the MHC on other chromosomes. In contrast, at least a portion of the NK cell receptor molecules in the chicken are encoded by loci near and within the MHC itself. To gain insight into how MHC and NK receptor genes evolve and interact in the chicken, we are analyzing genes found within *B* and *Rfp-Y*, the two genetically unlinked MHC gene clusters on chicken chromosome 16. Sequencing has been completed for two Jungle Fowl BAC clones representing portions of *B* and *Rfp-Y*. We have identified nearly 40 gene sequences within 200 kb. Among these are ten NK lectin-like sequences within the *Rfp-Y* region that are intermingled with a family of sequences for nine non-classical MHC class I loci. Other loci within the *B* region include a previously unmapped lectin locus, as well as additional unexpected loci. The close proximity of NK lectin-like and class I loci within *Rfp-Y* may be evidence that these loci co-evolve providing arrays of alleles that work together as units effective in immune defense. A consequence of this may be the ease with which associations can be found in the chicken between particular MHC haplotypes and infectious disease. Supported by NSF and USDA NRICGP.

Key Words: Gene mapping, Innate immunity, NK lectin-like receptors, Non-classical MHC class I loci