

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

7 Non-MHC alloantigen genes affecting immunity. W. E. Briles*, Northern Illinois University, DeKalb, IL 60115.

An alloantigen is a genetically determined cell-surface molecule detected by specific antisera. An identifying letter has been assigned to each genetic locus responsible for the eleven distinct families of alloantigens - *A, C, D, E, H, I, J, K, L, P,* and *R*. The genes of each system segregate independently of the other systems, except that the *A* and *E* are very closely linked (0.5% crossing over). Selection experiments over numerous generations have revealed distinct changes in gene frequency of the *A-E* alloantigens, suggesting immune responses associated with susceptibility to coccidiosis, antibody response to sheep red blood cells (SRBC), regression of Rous sarcomas, and selection for size of the bursa of Fabricius. Immune response effects of the *C* system of alloantigen genes are indicated by distinct gene frequency changes following selection for response to SRBC, selection for size of bursa of Fabricius, differential development of Rous sarcoma tumors, and macrophage nitrite production after LPS stimulation. Immune response effects of the *D* system of

antigens are indicated by data from genetic selection for response to immunization with SRBC, selection for bursa size, and macrophage nitrite production following LPS stimulation. Immune response effects of the *I* system genes are indicated by distinct gene frequency changes in lines selected for bursa size, response to SRBC, selection for egg weight and within family comparisons for macrophage nitrite production. Effects of the *L* system, consisting of only two alleles, are indicated by the gene frequency changes following selection for bursa size, observed fertility effects, and direct comparison of genotypes within families for monocyte phagocytosis, IL-6 production, outcome of Rous sarcomas, and immune responses to SRBC and *Brucella abortus*. Genotypes of the *P* alloantigen system were directly compared within families of fully pedigreed chicks with significant differences for monocyte phagocytosis and macrophage nitrite production. Simultaneous segregation of eight systems in progeny of a single-cross are available to provide critical evaluation of immune response.

Key Words: Alloantigen, Genotypes, Haplotype, Immune effect

Systems for Understanding the Immune System Role in Disease Processes

8 Cytokine regulation of local host immune responses to *Eimeria*. H. Lillehoj*, USDA-ARS, Beltsville, MD.

Parasitic infections usually stimulate a number of immunological defense mechanisms, namely both antibody- and cell-mediated. The particular effect of the response depends upon the specific parasite and stage of infection. Our recent studies on local host immunity to *Eimeria* clearly indicate that intricate and complex interactions of host local cell-mediated immunity and parasites determine the outcome of the host response to coccidiosis. High-throughput gene expression profiling and real-time PCR have been applied to analyze underlying immune mechanisms controlling disease resistance/susceptibility to coccidiosis. The role of various cytokines whose expression increase and decrease in response to coccidia invasion will be discussed.

Key Words: Coccidiosis, Gene expression profiling, Cytokines, Local immunity, Chickens

9 Immune modulation of the pulmonary hypertensive response to bacterial lipopolysaccharide (LPS, endotoxin) in broilers. R. F. Wideman, Jr.* and M. E. Chapman, University of Arkansas, Fayetteville AR 72701.

The lungs of broilers are constantly challenged with LPS that can activate leukocytes and trigger thromboxane (Tx)- and serotonin (5HT)-mediated pulmonary vasoconstriction leading to pulmonary hypertension. Among broilers from a single genetic line, some individuals respond to LPS with large increases in pulmonary arterial pressure, whereas others fail to exhibit any response to the same supra-maximal dose of LPS (Wideman et al., 2001; Wang et al., 2002a,b). This extreme variability in the pulmonary hypertensive response to LPS appears to reflect variability in the types or proportions of chemical mediators released by leukocytes. Our research has confirmed that Tx and 5-HT are potent pulmonary vasoconstrictors in broilers, and that broilers hatched and reared together consistently exhibit pulmonary hypertension after i.v. injections of Tx or 5-HT (Wideman et al., 1999; Chapman and Wideman, 2002). Previous in vitro studies conducted using macrophages from different lines of chickens demonstrated innate variability in the LPS-stimulated induction of nitric oxide synthase (iNOS), followed by the onset of an LPS-refractory state (Chang et al., 1996; Hussain and Qureshi, 1997). The NOS enzyme converts arginine to citrulline and nitric oxide (NO). It is known that NO produced by endothelial NOS (eNOS) serves as a key modulator of flow-dependent pulmonary vasodilation (Wideman et al., 1995, 1996), and it is likely that NO generated by iNOS also can function as a vasodilator. Accordingly, it is our hypothesis that broilers exhibit a minimal pulmonary hypertensive response to LPS when their leukocytes innately generate more vasodilator (NO) than vasoconstrictor (Tx, 5-HT) during an LPS challenge. Indeed, inhibiting NOS with L-NAME (and thus inhibiting NO production) modestly increased the baseline pulmonary arterial pressure and dramatically increased the pulmonary hypertensive response to LPS in all broilers evaluated. Innate differences in the effect of LPS on the pulmonary vasculature may parallel genetic differences

in susceptibility of broilers to pulmonary hypertension syndrome (PHS, ascites).

Key Words: Ascites, Pulmonary hypertension, Vasoconstriction, Nitric oxide

10 Major histocompatibility (*B*) complex control of responses against Rous sarcomas. R. L. Taylor, Jr.*, Department of Animal and Nutritional Sciences, University of New Hampshire, Durham, NH 03824.

The chicken major histocompatibility (*B*) complex (MHC), affects disease outcome after pathogen infections significantly. One of the best characterized systems of MHC control is the response to the oncogenic retrovirus, Rous sarcoma virus (RSV). Early experiments found that selection altered the tumor growth pattern, either regression or progression. Furthermore, the data suggested genetic control by one or, at best, a few genes. Simultaneous reports defined the essential role of the *B* complex in determining Rous sarcoma outcome. Particular MHC genotypes regressed RSV tumors whereas other MHC genotypes progressed these tumors. Analysis of inbred lines and their crosses, congenic lines as well as noninbred populations has revealed the anti-RSV response of many *B* complex haplotypes. Disparity in tumor growth among lines identical at the MHC but differing in their background genes suggested a contribution of non-MHC genes in the determination of tumor fate. Genetic complementation in tumor growth, characterized by a improved response in heterozygotes, has been demonstrated for both MHC and non-MHC genes. RSV-induced tumor expansion reflects tumor cell proliferation as well as viral replication generating new tumor cells. The *B* complex also controls tumor growth induced by a subviral DNA construct encoding the RSV *v-src* oncogene without other viral sequences. Two other characteristics, immunity to a second tumor induction and metastasis, exhibit MHC control. Genotypes that regressed either RSV or *v-src* DNA primary tumors had enhanced protection against subsequent homologous challenge. In addition, regressor *B* complex genotypes had lower metastatic tumor dissemination to distant sites compared with progressor types. Together, the data indicate that MHC control of RSV-induced tumor fate is strongly defined by the response to a *v-src*-determined function. That function may be a tumor-specific antigen recognized by the immune system. Differential RSV tumor outcomes among various *B* genotypes may include immune influences on viral replication.

Key Words: *B* complex, Rous sarcoma virus, *v-src*, oncogene