J. Vet. Sci. (2004), **5**(4), 279–288

JOURNAL OF Veterinary Science

Review

Application of biotechnological tools for coccidia vaccine development

Wongi Min[1,2], Rami A. Dalloul[1], Hyun S. Lillehoj[1,*]

[1]*Animal Parasitic Diseases Laboratory, Animal and Natural Resources Institute, U. S. Department of Agriculture, Beltsville, MD. 20705, USA*
[2]*Department of Animal Science & Technology, Sunchon National University, Suncheon 540-742, Korea*

Coccidiosis is a ubiquitous intestinal protozoan infection of poultry seriously impairing the growth and feed utilization of infected animals. Conventional disease control strategies have relied on prophylactic medication. Due to the continual emergence of drug resistant parasites in the field and increasing incidence of broiler condemnations due to coccidia, novel approaches are urgently needed to reduce economic losses. Understanding the basic biology of host-parasite interactions and protective intestinal immune mechanisms, as well as characterization of host and parasite genes and proteins involved in eliciting protective host responses are crucial for the development of new control strategy. This review will highlight recent developments in coccidiosis research with special emphasis on the utilization of cutting edge techniques in molecular/cell biology, immunology, and functional genomics in coccidia vaccine development. The information will enhance our understanding of host-parasite biology, mucosal immunology, and host and parasite genomics in the development of a practical and effective control strategy against *Eimeria* and design of nutritional interventions to maximize growth under the stress caused by vaccination or infection. Furthermore, successful identification of quantitative economic traits associated with disease resistance to coccidiosis will provide poultry breeders with a novel selection strategy for development of genetically stable, coccidiosis-resistant chickens, thereby increasing the production efficiency.

Key words: Chicken, *Eimeria*, coccidiosis, vaccine, biotechnology

Introduction

Intestinal infections such as coccidiosis, salmonellosis, and cryptosporidiosis are prevalent in commercially bred chickens and inflict severe economic losses on the poultry industry [78,85,88]. Many avian diseases are currently controlled by chemoprophylaxis in ways that promote development of drug resistant pathogens and at great cost to the poultry industry. Prophylactic drug usage also creates unnecessary anxiety in a consuming public already concerned with chemical residues in food. Consequently, the past two decades have witnessed great interest in alternative strategies to control avian diseases. Among the more promising of these are development of new vaccines based upon in depth analysis of the genomes and proteomes of multiple *Eimeria* species, and the characterization of host effector molecules which impact the development of resistance to infection with *Eimeria* species.

Because the life cycle of *Eimeria* comprises intracellular, extracellular, asexual, and sexual stages, it is not surprising that host immunity is also complex and involves many facets of non-specific and specific immunity, the latter encompassing both cellular and humoral immune mechanisms [82,86,128]. Chickens infected with *Eimeria* produce parasite-specific antibodies in both the circulation and mucosal secretions, but humoral immunity plays only a minor role in protection against this disease. Rather, studies conducted at our laboratory implicate cell mediated immunity (CMI) as the major factor conferring resistance to coccidiosis. It is anticipated that increased knowledge on the interaction between parasites and host will stimulate the development of novel immunological and molecular biological concepts in the control of intestinal parasitism crucial for the design of new approaches against coccidiosis.

Life cycle

Eimeria are obligate intracellular parasites that carry out their life cycle in epithelial cells of the intestinal mucosa, often causing serious damage to the physical integrity of the gut. Oocysts ingested by feeding birds excyst to generate invasive sporozoites and sporogony ensues within 24 hours. Sporozoites are first seen within intestinal intraepithelial lymphocytes (IELs), primarily $CD8^+$ cells, shortly after invasion [116]. Sporozoites undergo merogony resulting in the release from one sporozoite of about 1,000 merozoites,

*Corresponding author
Tel: +1-301-504-8771; Fax: +1-301-504-5103
E-mail: hlilleho@anri.barc.usda.gov

sometimes repeating this stage 2-4 times before merozoites differentiate into the sexual stages, gamonts and gametes. Microgametes (male) fertilize macrogametes (female) to produce oocyst encased by a thick wall impervious to the harshest of environmental conditions and subsequently excreted. Once outside the host, oocysts sporulate and can remain viable for long periods of time before being ingested and starting the life cycle over again.

Host cell invasion and parasite proteins associated with invasion

Invasion of host epithelial cells or cultured cells by sporozoites follows a conserved but complex scheme initiated by contact between the anterior end of the sporozoite and the cell surface [8,38]. The initial contact is followed by internalization of the membrane eventually enclosing the parasite within a vacuole. The invasion is driven by the parasite and is entirely dependent on gliding motility thought to be actin-myosin dependent. Apical organelles of the parasite, the rhoptries and micronemes, are involved in the invasion process. Micronemes are excytosed during initial contact with the host cell and provide the formation of a moving junction with the host cell membrane. The rhoptries are excytosed while the parasitophorous vacuole is expanding and are integrated into the vacuole membrane. In some apicomplexans, dense granules are also secreted into the vacuolar space.

Some of the parasite proteins involved in host cell invasion have begun to be characterized by using antibodies [108,118] and by selective labeling with ricin [52]. Micronemes from *E. tenella* contain at least 10 major proteins secreted into culture medium during cell invasion [14,17]. Rhoptries from *Eimeria* contain at least 60 independent polypeptides that can be resolved by 2D-electrophoresis. However, rhoptries from three species of *Eimeria* share few antibody cross-reactive epitopes [113]. Sequencing of genes coding for organelle proteins has shown several domains and motifs conserved among the apicomplexa, particularly the microneme proteins [114]. Several antibodies against surface proteins of *E. tenella* and *E. acervulina* sporozoites blocked invasion of sporozoites into host cells *in vitro* [108,118]. For analysis of protective immunity against *Eimeria in vivo*, chicken monoclonal antibodies with chicken B cell line were made [108] and recombinant single chain variable fragment (scFv) antibodies were constructed to circumvent the problems associated with chicken hybridoma [65,98]. The ability to develop an *in vitro* culture system for other *Eimeria* species would facilitate genomic analysis of developmental stages of *Eimeria* species such as *E. acervulina* and *E. maxima*. High throughput expressed sequence tag (EST) sequencing of *Eimeria* will facilitate functional genomics studies of *Eimeria* to identify parasite genes involved in host invasion.

Immunopathology and pathobiology

Most major enteric parasites, including coccidia, invade the intestinal mucosa and induce a certain degree of epithelial cell damage and inflammation. Extensive damage leads to diarrhea, dehydration, weight loss, rectal prolapse, dysentery, serious clinical illness and, at times, mortality [28]. Reduced weight gains and increased feed conversions are major characteristics of avian coccidiosis, and are the main contributors to the cost of this disease to the poultry industry. Nutrient malabsorption can account for some of the reduced weight gain [106]. However, reduced feed intake due to anorexia is also involved. Klasing *et al.* [66] was among the first to show that growth depression in chickens could be mediated by inflammatory cytokines such as IL-1, and was related to decreased feed intake. Recent investigations [58-60,62,63] have strengthened the concept that the immune, neuroendocrine, and central nervous systems are linked through networks of common receptors and ligands, and that they work together to modulate disease resistance, metabolism, and growth. In particular, it is now known that the expression of leptin, a peptide that homes to the hypothalamus and causes depressed feed intake and reduced energy expenditure, is upregulated by inflammatory cytokines. Finck and Johnson [43] have suggested that hyperleptinemia induced by cytokines is an integral part of the acute phase response, and necessary for comprehensive immunocompetence. Chicken leptin has been cloned [103] and its quantitation methods have been developed [104,105]. Following the leptin response during infections with the separate *Eimeria* species should provide insight into the regulation of weight gain during coccidia infections as well as measuring markers for muscle protein turnover, 3-methylhistidine [41] and growth, IGF-1 [20].

Host defense mechanisms, acute phase proteins and oxidative stress

Cells of the host immune system, commonly macrophages, produce superoxide as a product of phagocytosis and nitric oxide when stimulated by interferon (IFN) or other cytokines. Both superoxide and nitric oxide, as well as peroxynitrite, a reaction product, are toxic to parasites. In order to survive, parasites must detoxify these reactive oxygen and nitrogen species. Some utilize antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase. These enzymes are electrophoretically distinct from homologous host enzymes [54]. Of the avian *Eimeria*, only unsporulated oocysts of *E. tenella* have high levels of SOD [97]. The sporozoites have only low levels. The existence of glutathione-based defense mechanisms for avian *Eimeria* has not been investigated as yet, although they are present in parasites such as trypanosomes [29]. However, *E. tenella*, and presumably other avian *Eimeria*

species, have a mannitol cycle which may serve the purpose of keeping the parasite protected from an oxidative environment [109]. Knowledge of the intermediary metabolism of avian *Eimeria* has come mainly from analyses of *E. tenella* that develops in the relatively anaerobic environment of the intestinal cecum, and some investigators have concluded that avian *Eimeria* are facultative anaerobes [29].

Alpha-1-acid glycoprotein [22], hemopexin [48] and ovotransferrin [53] also are known to be acute phase response proteins in chickens. These proteins may vary characteristically with the infecting species of *Eimeria* and could serve as biomarkers of resistance and susceptibility. Some metal binding proteins (metallothionein and ceruloplasmin) are apparently differentially elevated in acute infections with *E. acervulina* and *E. tenella* [102].

There is an increased recognition of the importance of oxidative stress as an initiator of signal transduction in biological processes [42,44,111]. Externally or internally generated free radicals such as superoxide and nitric oxide [18,100], or changes in the redox potential of cells [37,67, 101,110], can lead to production of second messengers and transcription factors that up-regulate genes expressing antioxidant factors, including enzymes that counteract the oxidative stress [42]. Oxidative stress is an important regulator of immunity [30,36,70] and an important component of host-parasite interactions [7,40,54,99]. Production of free radical species accompanies infection by murine coccidia and all species of avian coccidia [1-3]. Furthermore, recent experiments showed that major alterations in whole body thiol balance, as illustrated by significant reductions in both reduced and oxidized whole blood glutathione, occur from days 3-10 post-infection with *E. acervulina* and *E. tenella*. Reduced glutathione is one of the major cellular components maintaining a reduced intracellular environment in normal cells [5]. Expression of enzymes that control glutathione and other cellular antioxidant components are regulated by cytokines and other biological messenger molecules elaborated during the immune response. Application of genomic analyses over time courses of infection to investigate the enzymes catalyzing both the oxidative response to parasite invasion as well as the host enzymes that counteract oxidative stress could provide important clues to innate resistance and development of immunity to coccidiosis.

Gut-associated lymphoid tissues (GALT)

The mucosal immune system is composed of the mucosal-associated lymphoid tissues (MALT) of the nasal passage, bronchial organs, mammary glands, genital tract, and gut-associated lymphoid tissues (GALT) [13,94,95]. The most important role of the MALT is to destroy invading pathogens at their port of entry to prevent dissemination and systemic infection throughout the host. This function is accomplished in a number of different ways. Non-specific barriers such as gastric secretions, lysozyme and bile salts, peristalsis, and competition by native microbial flora provide an important component of the first line of defense in the MALT [107]. Specific defense mechanisms are mediated by antibodies and lymphocytes. The specific mechanisms for elimination of harmful pathogens involve complex interactions among humoral and cellular immunity utilizing both antigen specific and antigen independent processes that have evolved to detect and neutralize invading microorganisms.

More than half of the total lymphocyte pool of the MALT is contained within the GALT. Histologically, the outer layer of the GALT consists of epithelial cells and lymphocytes situated above the basement membrane. Beneath the basement membrane is the lamina propria, also containing lymphocytes, and the submucosa. In chickens, a variety of specialized lymphoid organs (Peyers patches, caecal tonsils and bursa of Fabricius) and cell types (epithelial, lymphoid, antigen presenting and natural killer cells) have evolved in the GALT to defend against harmful pathogens. Other cell types in the GALT include macrophages, mast cells, fibroblasts, and dendritic cells. All of these cells are known to secrete and respond to cytokines. Cellular communication networks within the GALT, important for development of protective immunity, are bi-directional with lymphocytes secreting and responding to cytokines that stimulate or inhibit the activities of other lymphocytes and non-lymphoid, resident cells.

The mucosal immune system contains a number of unique cell types reflective of its evolution as the first line of immune defense [6]. T and B lymphocytes and plasma cells are located in the mucosa of the small and large intestines. T cells are predominantly $CD4^+$ memory/effector T cells while B cells and plasma cells are largely of the IgA isotype. Within the intestinal mucosa, lymphocytes are present in two anatomic compartments, the epithelium (IELs) and lamina propria (lamina propria lymphocytes) [9]. As with mammals, chicken IEL T cells can be phenotypically separated into $CD4^+$ and $CD8^+$ subpopulations [23]. Molecular complexes similar to human and murine CD3, CD4, and CD8 antigens have been identified on chicken IELs [23,73]. The ontogeny of T cells bearing the two different T cell receptor (TCR) molecules has been studied [15,119].

Adaptive immune responses to *Eimeria*

In general, the GALT serves three functions in host defense against enteric pathogens, processing and presentation of antigens, production of intestinal antibodies and activation of CMI. The role of parasite specific antibodies both in serum and mucosal secretions has been

extensively studied in coccidiosis [46,47,72,115]. In infected chickens, production of specific antibodies, IgA and IgM in particular, was always significantly greater in parasitized areas of the intestine compared with areas devoid of parasites [46]. However, the ability of antibodies to limit infection is minimal, if any, since agammaglobulinemic chickens produced by hormonal and chemical bursectomy are resistant to reinfection with coccidia [71]. Nevertheless, IgA may attach to the coccidial surface and prevent binding to the epithelium by direct blocking, steric hindrance, induction of conformational changes, and/or reduction of motility. Mucosal IgA responses were regulated by T helper cells and cytokines [126].

It is clearly documented that CMI mediated by antigen specific and non-specific activation of T lymphocytes, natural killer (NK) cells and macrophages plays the major role in protection against coccidia [12,21,86,128]. The importance of T cells in acquired immunity to coccidia has been well documented [82]. For example, changes in intestinal T cell subpopulations in the duodenum following primary and secondary *E. acervulina* infections have been investigated and correlated with disease [75,79,82]. Following secondary infection, a significantly higher number of $CD8^+$ IELs was observed in SC chickens, which manifested a lower level of oocyst production compared to TK chickens. In summary, these results identified variations in T cell subpopulations in the GALT as a result of coccidia infection and suggested that increase in $\alpha\beta TCR^+CD8^+$ IELs in SC chickens may contribute to their enhanced immunity to *Eimeria* compared with TK chickens [77,80,81]. The immunological basis for the genetic difference in disease resistance has been addressed by recent studies, which showed different kinetic and quantitative response in local cytokine production between SC and TK chickens following *Eimeria* infection [130,131]. IFN-γ mRNA in caecal tonsils was higher in SC chickens following primary infection with *E. acervulina* [26]. Zhang *et al.* [132,133] investigated the effect of a cytokine with tumor necrosis factor activity on the pathogenesis of coccidiosis in SC and TK chickens. In summary, these results all emphasize the importance of CMI in protective immunity to coccidiosis.

Cytokine production during CMI to coccidiosis

In contrast to the plethora of mammalian cytokines, only a few chicken homologues have been described, the mains ones being IFN-γ, TGF, TNF, IL-2 and IL-15 [82,89]. T lymphocytes and macrophages are the most likely sources of cytokine production in the intestine [79]. Intestinal lymphocytes have been observed in direct contact with parasitized epithelial cells promoting the hypothesis that they are producing cytokines and thereby modulating the immune response [80,116,117]. The availability of recombinant chicken IFN-γ and its monoclonal antibodies has led to a better understanding of its physiologic and immunologic roles in chicken coccidiosis [83,90,91,129]. Administration of exogenous recombinant IFN-γ to chickens significantly hindered intracellular development of *Eimeria* parasites and reduced body weight loss [87]. When chicken fibroblast cells transfected with the IFN-γ gene were infected with *E. tenella* sporozoites, significant reductions in parasite intracellular development occurred although the ability of parasites to bind and invade host cells was not affected [87]. Although the biological function of this cytokine in the intestine requires further investigation, these results indicate a major role of CMI in protective immunity to pathogens in this organ.

Application of poultry genomics for control of coccidiosis

Tremendous success in the improvement of commercial chicken growth, reproduction and feed efficiency has been accomplished using classical genetic breeding techniques. However, selection of commercial poultry stocks for improved disease resistance using similar breeding techniques has been unsuccessful due to technical difficulties [45]. Although selection based on progeny tests may be used to avoid this negative impact, as demonstrated by selection of broiler strains with enhanced antibody responsiveness to *Salmonella enteritidis* [61], this is a labor-intensive, time consuming and costly approach. Moreover, lack of a clear understanding of the mechanisms of protective immunity against most avian diseases makes genetic selection of stocks with enhanced disease resistance very difficult [84]. DNA marker technology avoids many of these problems, making it easier to select animals with superior performance for resistance to particular diseases of commercial importance. In the DNA marker approach, phenotypic traits for disease resistance are measured in genetically diverse animals challenged with the pathogen of interest. DNA marker(s) associated with disease resistance are identified in particular genotypes and subsequently used for marker-assisted selection of breeding stocks.

Most of the economically important traits (quantitative traits) of food animals are regulated by multiple genes that manifest different effects and are continuously distributed in the population. The loci affecting these traits are referred to as quantitative trait loci (QTL) [4,92,93]. With DNA marker technology and statistical methodology, it is possible to map QTL on chromosomes. DNA marker-based methods have had a significant impact on both gene mapping and animal breeding [35]. Genetic mapping using DNA markers that cover the entire genome, with defined intervals between the markers is called whole genome scanning. Candidate genes that potentially affect traits of interest and are positively correlated with QTL can thus be mapped on the genome. To map QTL efficiently, a linkage map with high marker

density is required. Bumstead and Palyga [16] reported the first DNA marker linkage map of the chicken genome. Currently, more than 1,800 DNA-based genetic markers are available for chicken genotyping [51]. A large number of these markers have been mapped to chicken linkage groups [24,25,31-33,50,51,68,69]. The current chicken linkage map covers more than 95% of the entire genome and provides sufficient marker density for QTL mapping with an average interval of less than 20 cM [50]. QTL affecting animal growth [49,122], feed efficiency [123], carcass traits [124], and Marek's disease [120,121,125,127] have been reported.

In general, larger population sizes increase QTL detection power. Groenen *et al.* [50] suggested that with 100% genome coverage, the preferred distance between adjacent markers is 20 cM or less to map loci affecting quantitative traits in initial genetic mapping studies. Given the size of the chicken genome, approximately 200 evenly spaced markers are needed to cover the entire genome. The distance (m) of 20 cM is equal to 0.165 of the recombination fraction (r) according to Haldanes mapping function, $r = 0.5\ (1 - e^{-2m})$. For example, in the broiler chickens used in our study, the marker MCW0058 affecting animal growth (selected trait) is 20 cM from the marker LEI0101 affecting coccidiosis resistance (non-selected trait). By computer simulation, a 50 cM marker interval was found to be optimal or close to optimum for initial studies in a variety of experimental designs, if experimental cost is a limiting factor [34].

The chicken genome comprises 39 pairs of chromosomes, 8 pairs of cytologically distinct chromosomes, one pair of sex chromosomes (Z and W), and 30 pairs of small, cytologically indistinguishable microchromosomes. The size of chicken genome is estimated to be 1.2 billion base pairs [11] and approximately 3,500 to 4,000 cM in genetic length. Therefore, 1 cM is equivalent to approximately 350 kb. There are several high capacity vectors available to clone chicken genomic DNA. These include cosmids (maximum insert size = 30-45 kb), bacteriophage P1 (70-100 kb), P1 artificial chromosomes (130-150 kb), bacterial artificial chromosomes (BAC, 120-300 kb), and yeast artificial chromosomes (250-400 kb). Among these, BAC are the most attractive vectors because they are stable, capable to propagate very large DNA fragments and easy to manipulate. Recently, two chicken genomic DNA libraries were constructed at the Texas A&M BAC Center. Both were derived from the Red Jungle fowl (UCD 001) with the intention of maximizing genetic heterogeneity in expressed clones. The first library was derived from *Hin*d III-digested genomic DNA and inserted into the BAC vector pECBAC1. It contains 49,920 clones representing 5.4-times genomic coverage. The average insert size of this library was estimated to be 130 kb. The second library was created from *Bam* HI partial digests of UCD 001 genomic DNA cloned into pBeloBAC11. Its average insert size was estimated to be 150 kb. This library is also maintained at the University of Michigan by Dr. Jerry Dodgson (Coordinator for NAGRP/NRSP-8). For these studies, both libraries will be used to construct BAC clone contigs covering the chromosomal region of interest.

DNA microarray for gene expression studies

In addition to DNA marker and cloning technologies, DNA microarray is another revolutionary tool for genomic study of interesting traits. By immobilizing thousands of DNA sequences in individual spots on a solid phase, DNA microarray allows simultaneous analysis of a large number of genes in a single step, thereby identifying genes whose expression levels are altered during natural biological processes or experimental treatments or vary due to genetic differences [39]. In one approach, the sample of interest, such as mRNA isolated from a certain tissue, is used to synthesize cDNA labeled with colored substances (e.g., fluorescent dyes like Cyanine). The labeled cDNA probes (both Cy3 and Cy5) are then hybridized to the array at 42°C for 16-18 h. and a post-hybridization image is scanned to capture fluorescence images using a ScanArray 4000 Microarray Analysis System (GSL Lumonics) and is analyzed using ScanAlyze software developed at Stanford University. The color density of individual nucleic acid species reflects the relative amount of labeled cDNA hybridized to the DNA immobilized at the known position of the array. By comparing samples tested in well-controlled conditions, change of expression levels of individual genes can be detected. The DNA sequences immobilized on an array are usually produced by PCR from genes whose sequences are partially or completely known. This technique has been widely used to detect gene mutations and polymorphisms, gene expression profiling, genetic linkage, sequence analysis, and single nucleotide polymorphism-based tests [96]. While only a small number of chicken genes have been cloned and completely sequenced, more than 5,000 chicken ESTs from mitogen-activated chicken T cell and macrophage cDNA libraries [112] are currently available for designing DNA microarrays. Using EST sequences from activated T-cell cDNA library, several genes associated with immune response have been identified using DNA array.

Several methods exist to quantify microarray signals and the best method to use is often based on how well each measurement correlates with the amount of DNA probe hybridized to each printed spot. Quantitation can be based on the following signal parameters: total (sum of intensity values of all pixels in a spotted area), mean, median, mode (most likely intensity value), volume (difference between signal mean and background multiplied by signal area), intensity ratio of two colors, or correlation ratio [134]. The best method for a particular experimental design can be determined by analysis of duplicate experiments. Data

normalization and transformation are other important processes to improve the quality of array data [134]. Many analysis methods have been implemented in commercial software. Differences in gene expression detected by DNA microarray have been demonstrated to be highly correlative with the results of Northern blot analysis [10].

Vaccines against *Eimeria*

Identification of parasite life cycle stages and development-specific antigens inducing protective immunity is a critical step in recombinant protein vaccine development. In the case of *Eimeria*, recombinant forms of both parasite surface antigens and internal antigens have been investigated as vaccine candidates. Sporozoites are the preferred parasitic form for preparation of recombinant vaccines because they are relatively easy to obtain and blocking their activities should theoretically prevent infection. Cell surface antigens are logical components of vaccines because of their direct role in host-parasite interactions. cDNAs encoding a 22 kDa surface protein and EAMZp30-47 protein of *E. acervulina* sporozoites were cloned and expressed [55,56,57]. The recombinant protein (MA1) induced significant *in vitro* activation of T lymphocytes obtained from chickens inoculated with *E. acervulina* [74]. A cDNA (MA16) from *E. acervulina* encoding an immunogenic region of a surface antigen shared between sporozoites and merozoites was cloned, expressed in *E. coli* and shown to activate T lymphocytes *in vitro* from *E. acervulina* immune chickens [19]. Intramuscular immunization with a recombinant p250 surface antigen of *E. acervulina* merozoites or oral inoculation with live *E. coli* expressing p250 resulted in antigen specific *in vitro* T cell and humoral responses and conferred significant reduction in mucosal parasitism [64,76]. Vaccination with *E. coli* expressing a recombinant protein is more effective than immunization with the protein alone since bacteria growing in the intestine continuously express the recombinant protein thus providing antigenic stimulation over an extended period of time.

DNA vaccines employ genes encoding immunogenic proteins of pathogens rather than the proteins themselves. They are administered directly in conjunction with appropriate regulatory elements (promoters, enhancers) permitting the encoded protein to be expressed in its native form and thereby recognized by the host's immune system in a manner that simulates natural infection. DNA vaccination requires gene transfer and expression of the antigen in tissues accessible to the immune system such as the skin, muscle or mucosal surfaces. Lillehoj *et al.* [87] observed immune protection manifested by significantly reduced fecal oocyst shedding in chickens vaccinated subcutaneously with a cDNA encoding an *E. acervulina* protein (3-1E). Further protection was obtained when the 3-1E cDNA was administered in conjunction with cDNAs encoding chicken IFN-γ or IL-2 [27,87]. These results raise the exciting possibility of using IFN-γ and/or IL-2 immunoprophylactically to control coccidiosis in commercial poultry flocks. In spite of the advantages of DNA vaccines over conventional vaccines, the negative side factors such as the genetic background of the recipient must be considered [72,78,85].

Acknowledgments

This project was partially supported by National Research Initiative grant 2004-01154 from the CSREES, United States Department of Agriculture.

References

1. **Allen PC.** Nitric oxide production during *Eimeria tenella* infections in chickens. Poult Sci 1997, **76**, 810-813.
2. **Allen PC.** Production of free radical species during *Eimeria maxima* infections in chickens. Poult Sci 1997, **76**, 814-821.
3. **Allen PC, Lillehoj HS.** Genetic influence on nitric oxide production during *Eimeria tenella* infections in chickens. Avian Dis 1998, **42**, 397-403.
4. **Almasy L, Blanqero J.** Multipoint quantitative-trait linkage in general pedigrees. Am J Human Genet 1998, **62**, 1198-1211.
5. **Anderson ME.** Glutathione: an overview of biosynthesis and modulation. Chem Biol Interact 1998, **24**, 1-14.
6. **Arnaud-Battandier F, Lawrence EC, Blaese RM.** Lymphoid populations of gut mucosa in chickens. Digest Dis Sci 1980, **25**, 252-59.
7. **Assreuy J, Cunha FQ, Epperlein M, Noronha-Dutra A, O'Donnell CA, Liew FY, Moncada S.** Production of nitric oxide and superoxide by activated macrophages and killing of *Leishmania major.* Eur J Immunol 1994, **24**, 672-676.
8. **Augustine PC.** Molecular interactions of cultured turkey kidney cells with specific antigens of Eimeria adenoeides sporozoites. Proc Exp Biol Med 1989, **191**, 30-36.
9. **Befus AD, Johnston N, Leslie GA, Bienenstock J.** Gut-associated lymphoid tissue in the chicken. I. Morphology, ontogeny, and some functional characteristics of Peyer's patches. J Immunol 1980, **125**, 2626-2632.
10. **Bitiner M, Chen YY, Amundson AA, Khan J, Formace Jr. AJ, Dougherty ER, Meltzer PS, Trent JM.** Obtaining and evaluating gene expressing profile with cDNA microarrays. In: Suhai S. (ed), Genomics and Proteomics: Functional and Computational Aspects. pp. 5-25. Kluwer Academic/ Plenum Publishers, New York, 2000.
11. **Bloom SE, Delaney ME, Muscarella DE.** Manipulation of the avian genome, pp. 39-59, CRC Press, Boca Raton, 1993.
12. **Brandtzaeg P, Baklien K, Bjerke K, Rognum TO, Scott H, Valnes K.** Nature and properties of the human gastrointestinal immune system, In: Miller K, Nicklin S. (eds.), Immunology of the Gastrointestinal Tract. pp. 1-85. CRC Press, Boca Raton, 1987.
13. **Brandtzaeg P, Halstensen TS, Kett K, Krajci P, Kvale D, Rognum TO, Scott H, Sollid LM.** Immunobiology and

immunopathology of human gut mucosa: humoral immunity and intraepithelial lymphocytes. Gastroenterology 1989, **97**, 1562-1584.

14. **Brown PJ, Billington KJ, Bumstead JM, Clark JD, Tomely FM.** A microneme protein from *Eimeria tenella* with homolog to Apple domains of coagulation factor XI n plasma pre-kallikrein. Mol Biochem Parasitol 2000, **107**, 91-102.
15. **Bucy RP, Chen CL, Cihak J, Losch U, Cooper MD.** Avian T cells expressing gd receptors localize in the splenic sinusoids and the intestinal epithelium. J Immunol 1988, **41**, 2200-2205.
16. **Bumstead N, Palyga J.** A preliminary linkage map of the chicken genome. Genomics 1992, **13**, 690-697.
17. **Bumsted J, Tomely F.** Induction of secretion and surface capping of microneme proteins in *Eimeria tenella*. Mol Biochem Parasitol 2000, **110**, 311-321.
18. **Burdon RH.** Oxyradicals as signal transducers. In: Forman HJ, Cadenas E. (eds.), Oxidative Stress and Signal Transduction. pp. 289-319. Chapman & Hall, New York, 1997.
19. **Castle MD, Jenkins MC, Danforth HD, Lillehoj HS.** Characterization of a recombinant *Eimeria acervulina* antigen expressed on both sporozoite and merozoite developmental stages. J Parasitol 1991, **77**, 384-390.
20. **Cerwinski SM, Cate JM, Francis G, Tomas F, Brocht DM, Mcmurtry JP.** The effect of insulin-like growth factor-1 (IGF-1) on protein turnover in the meat-type chicken (*Gallus domesticus*). Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 1998, **119**, 75-80.
21. **Chai JY, Lillehoj HS.** Isolation and functional characterization of chicken intestinal intra-epithelial lymphocytes showing natural killer cell activity against tumor target cells. Immunology 1988, **63**, 111-117.
22. **Chan J, Yu D**. One-step isolation of alpha-1-acid glycoprotein. Protein Expr Purif 1991, **2**, 34-36.
23. **Chan MM, Chen CL, Ager LL, Cooper MD.** Identification of the avian homologues of mammalian CD4 and CD8 antigens. J Immunol 1988, **140**, 2133-2138.
24. **Cheng HH, Levin I, Vallejo RL, Khatib H, Dodgson JB, Crittenden LB, Hillel J.** Development of a genetic map of the chicken with markers of high utility. Poult Sci 1995, **74**,1855-1874.
25. **Cheng HH.** Mapping the chicken genome. Poult Sci 1997, **76**, 1101-1107.
26. **Choi KD, Lillehoj HS, Zalenga DS.** Changes in local IFN-γ and TGF-β mRNA expression and intraepithelial lymphocytes following *Eimeria acervulina* infection. Vet Immunol Immunopathol 1999, **71**, 263-275
27. **Choi KD, Lillehoj HS.** Effect of chicken IL-2 on T-cell growth and function. Increased T cell response following DNA immunization. Vet Immunol Immunopathol 2000, **73**, 309-312.
28. **Cook GC.** Small-intestinal coccidiosis: an emergent clinical problem. J Infect 1988, **16**, 213-219.
29. **Coombs GH, Denton H, Brown SM, Thong K-W.** Biochemistry of the coccidia. Adv Parasitol 1997, **39**, 141-226.
30. **Crawford DR.** Regulation of mammalian gene expression by reactive oxygen species. In: Gilbert DL, Colton CA. (eds.), Reactive Oxygen Species in Biological Systems, An Interdisciplinary Approach. pp. 155-171. Kluwer Academic/ Plenum Publishers, New York, 1999.
31. **Crooijmans RPMA, van Kampen AJA, van Der Poel JJ, Groenen MAM.** New microsatellite markers on the linkage map of the chicken genome. J Hered 1994, **85**, 410-413.
32. **Crooijmans RPMA, van Der Poel JJ, Groenen MAM.** Functional genes mapped on the chicken genome. Anim Genet 1995, **26**, 73-78.
33. **Crooijmans RPMA, van Oers PAM, Strijk JA, van Der Poel JJ, Groenen MAM.** Preliminary linkage map of the chicken (*Gallus domesticus*) genome based on microsatellite markers: 77 new markers mapped. Poult Sci 1996, **75**, 746-754.
34. **Darvasi A, Soller M.** Optimum spacing of genetic markers for determining linkage between marker loci and quantitative trait loci. Theor Appl Genet 1994, **89**, 351-357.
35. **Dodgson JB, Cheng HH, Okimoto R.** DNA marker technology: a revolution in animal genetics. Poult Sci 1997, **76**, 1108-1114.
36. **Driscoll KE.** TNF alpha and MIP-2: role in particle-induced inflammation and regulation by oxidative stress. Toxicol Lett 2000,**15**, 177-183.
37. **Droge W, Schulze-Orthoff K, Mihm S, Galter D, Schenk H, Eck HP, Roth S, Gmunder H.** Function of glutathione and glutathione disulfide in immunology and immunopathology. FASEB J 1994, **8**, 1131-1138.
38. **Dubremetz JF, Garcia-Reguet N, Consell V, Fourmaux M N.** Apical organelles and host -cell invasion by Apicomplexa. Int J Parasitol 1998, **28**,1007-1013.
39. **Eisen MB, Brown PO.** DNA arrays for analysis of gene expression Methods Enzymol 1999, **303**, 179-205.
40. **Fernandes PD, Assreuy J.** Role of nitric oxide and superoxide in Giardia lamblia killing. Braz J Med Biol Res 1997, **30**, 93-99.
41. **Fetterer RH, Allen PC.** *Eimeria acervulina* infection elevated plasma and muscle 3-methythistidine in chickens. J Parasitol 2000, **86**, 783-791.
42. **Fialkow L, Downey GP.** Reactive oxygen intermediates as signaling molecules regulating leukocyte activation. In: Forman HJ, Cadenas E. (eds.), Oxidative Stress and Signal Transduction, pp. 200-235. Chapman & Hall, New York, NY. 1997.
43. **Finck BN, Johnson RW.** Tumor necrosis factor-alpha regulates secretion of the adipocyte-derived cytokine, leptin. Microsc Res Tech 2000, **50**, 209-215.
44. **Forman HJ, Cadenas E.** Oxidative Stress and Signal Transduction, pp. vii-ix. Chapman & Hall, New York, 1997.
45. **Gavora JS.** Disease genetics. In: Crawford RD. (ed), Poultry Breeding and Genetics, pp. 805-846. Elsevier, New York, 1990.
46. **Girard F, Fort G, Yvore P, Quere P.** Kinetics of specific immunoglobulin A, M and G production in the duodenal and caecal mucosa of chickens infected with *Eimeria acervulina* or *Eimeria tenella*. Int J Parasitol 1997, **27**, 803-809.
47. **Girard F, Pery P, Naciri M, Quere P.** Adjuvant effect of cholera toxin on systemic and mucosal immune responses in

chickens infected with *E. tenella* or given recombinant parasitic antigen per os. Vaccine 1999, **17**, 1516-1524.
48. **Grieninger G, Liang TJ, Beuving G, Goldfarb V, Metcalfe SA, Muller-Eberhard U.** Hemopexin is a developmentally regulated, acute phase plasma protein in the chicken. J Biol Chem 1986, **261**, 15719-15724.
49. **Groenen MAM, Crooijmans RPMA, Veenendaal A, van Kaam JBCHM, Vereijken ALJ, van Arendonk JAM, van der Poel JJ.** QTL mapping in chicken using a three generation full sib family structure of an extreme broiler x broiler cross. Anim Biotech 1997, **8**, 41-46.
50. **Groenen MAM, Crooijmans RPMA, Veenendaal A, Cheng HH, Siwek M, van der Poel JJ.** A comprehensive microsatellite linkage map of the chicken genome. Genomics 1998, **49**, 265-274.
51. **Groenen MAM, Cheng HH, Bumstead N, Benkel BF, Briles WE, Burke T, Burt DW, Crittenden LB, Dodgson J, Hillel J, Lamont S, de Leon AP, Soller M, Takahashi H, Vignal A.** A consensus linkage map of the chicken genome. Genome Res 2000, **10**,137-147.
52. **Gurnett AM, Dulski PM, Darkin-Rattray SJ, Carrington MJ, Schmatz DM.** Selective labeling of intracellular parasite proteins by using ricin. Proc Natl Acad Sci USA 1995, **92**, 2388-2392.
53. **Hallquist NA, Klasing KC.** Serotransferrin, ovotransferrin and metallothionein levels during the immune response in chickens. Comp Biochem Physiol Biochem Mol Biol 1994, **108**, 375-384.
54. **Hughes HP, Boik RJ, Gerhardt SA, Speer CA.** Susceptibility of Eimeria bovis and Toxoplasma gondii to oxygen intermediates and a new mathmatical model for parasite killing. J Parasitol 1989, **75**, 489-497.
55. **Jenkins MC, Lillehoj HS, Barta JR, Danforth HD, Strohlein DA.** *Eimeria acervulina*: cloning of a cDNA encoding an immunogenic region of several related merozoite surface and rhoptry proteins. Exp Parasitol 1990, **70**, 353-362.
56. **Jenkins MC, Augustine PC, Danforth HD, Barta JR.** X-irradiation of *Eimeria tenella* oocysts provides direct evidence that sporozoite invasion and early schizont development induce a protective immune response(s). Infect Immun 1991, **59**, 4042-4048.
57. **Jenkins MC, Castle MD, Danforth HD.** Protective immunization against the intestinal parasite *Eimeria acervulina* with recombinant coccidial antigen. Poult Sci 1991, **70**, 539-547.
58. **Johnson RW.** Inhibition of growth by pro-inflammatory cytokines: an integrated review. J Anim Sci 1997, **75**, 1244-1255.
59. **Johnson RW, Arkins S, Dantzer R, Kelley KW.** Hormones, lymphopoetic cytokines and the neuroimmune axis. Comp Biochem Physiol A Physiol 1997, **116**, 183-201.
60. **Johnson RW**. Immune and endocrine regulation of food intake in sick animals. Domest Anim Endocrinol 1998, **15**, 309-319.
61. **Kaiser MG, Wing T, Lamong SJ**. Effect of genetics, vaccine dosage, and postvaccination sampling interval on early antibody response to *Salmonella enteritidis* vaccine in broiler breeder chicks. Poult Sci 1998, **77**, 271-275.
62. **Kelly KW.** Cross-talk between the immune and endocrine systems. J Anim Sci 1988, **66**, 2095-2108.
63. **Kelly KW, Johnson RW, Dantzer R.** Immunology discovers physiology. Vet Immunol Immunopathol 1994, **43**, 157-165.
64. **Kim KS, Jenkins J, Lillehoj HS**. Immunization of chickens with live *Escherichia coli* expressing *Eimeria acervulina* merozoite recombinant antigen induces partial protection against coccidiosis. Infect Immun 1989, **57**, 2434-2440.
65. **Kim J-K, Min W, Lillehoj HS, Kim S-W, Sohn EJ, Song KD, Han JY.** Generation and characterization of recombinant scFv antibodies detecting *Eimeria acervulina* surface antigen. Hybridoma 2001, **20**, 175-181.
66. **Klasing KC, Laurin DE, Peng RK, Fry DM.** Immunologically mediated growth depression in chicks: Influence of feed intake, corticosterone and interleukin-1. J Nutr 1987, **117**, 1629-1637.
67. **Lawrence BP, Meyer M, Reed DJ, Kerkvliet NI.** Role of glutathione and reactive oxygen intermediates in 2,3,7,8-tetrachlorodibenzo-p-dioxin-indiced immune suppression in c57Bl/6 mice. Toxicol Sci 1999, **52**, 50-60.
68. **Levin I, Crittenden LB, Dodgson JB.** Genetic map of the chicken Z chromosome using random amplified polymorphic DNA (RAPD) markers. Genomics 1993, **16**, 224-230.
69. **Levin I, Santangelo L, Cheng H, Crittenden LB, Dodgson JB.** An autosomal genetic linkage map of the chicken. J Hered 1994, **85**, 79-85.
70. **Li N, Karin M.** Is NF-kappaB the sensor of oxidative stress? FASEB J 1999, **13**, 1137-1143.
71. **Lillehoj HS.** Effects of immunosuppression on avian coccidiosis: cyclosporin A but not hormonal bursectomy abrogates host protective immunity. Infect Immun 1987, **55**, 1616-1621.
72. **Lillehoj HS, Ruff MD.** Comparison of disease susceptibility and subclass-specific antibody response in SC and FP chickens experimentally inoculated with *Eimeria tenella*, *E. acervulina*, or *E. maxima*. Avian Dis 1987, **31**, 112-119.
73. **Lillehoj HS, Lillehoj EP, Weinstock D, Schat KA.** Functional and biochemical characterizations of avian T lymphocyte antigens identified by monoclonal antibodies. Eur J Immunol 1988, **18**, 2059-2065.
74. **Lillehoj HS, Ruff MD, Bacon LD, Lamont SJ, Jeffers TK.** Genetic control of immunity to *Eimeria tenella*. Interaction of MHC genes and non-MHC linked genes influences levels of disease susceptibility in chickens. Vet Immunol Immunopathol 1989, **20**, 135-148.
75. **Lillehoj HS.** Intestinal intraepithelial and splenic natural killer cell responses to eimerian infections in inbred chickens. Infect Immun 1989, **57**, 1879-1884.
76. **Lillehoj HS, Jenkins MC, Bacon LD.** Effects of major histocompatibility complex genes and antigen delivery on induction of protective mucosal immunity to *E. acervulina* following immunization with a recombinant merozoite antigen. Immunology 1990. **71**, 127-132.
77. **Lillehoj HS, Bacon LD.** Increase of intestinal intraepithelial lymphocytes expressing CD8 antigen following challenge infection with *Eimeria acervulina*. Avian Dis 1991, **35**, 294-301.

78. **Lillehoj HS, Trout JM.** Coccidia. A review of recent advances on immunity and vaccine development. Avian Pathol 1993, **22**, 3-21.
79. **Lillehoj HS.** Analysis of *Eimeria acervulina* induced changes in intestinal T lymphocyte subpopulations in two inbred chickens showing different levels of disease susceptibility to coccidia. Res Vet Sci 1994, **56**, 1-7.
80. **Lillehoj HS, Trout JM.** CD8+T cell-coccidia interactions. Parasitol Today 1994, **10**,10-14.
81. **Lillehoj HS, Trout JM.** Avian gut-associated lymphoid tissues and intestinal immune responses to *Eimeria* parasites. Clin Microbiol Rev 1996, **9**, 349-360.
82. **Lillehoj HS.** Role of T lymphocytes and cytokines in coccidiosis. Int J Parasitol 1998, **28**, 1071-1081.
83. **Lillehoj HS, Choi KD.** Recombinant chicken interferon-gamma-mediated inhibition of *Eimeria tenella* development *in vitro* and reduction of oocyst production and body weight loss following *Eimeria acervulina* challenge infection. Avian Dis 1998, **42**, 307-314.
84. **Lillehoj HS, Yun CH, Lillehoj EP.** Recent progress in poultry vaccine development against coccidiosis. Korean J Poult Sci 1999, **26**, 149-170.
85. **Lillehoj HS.** Review on vaccine development against enteric parasites *Eimeria* and *Cryptosporidium*. Jap Poult Sci 2000, **37**, 117-141.
86. **Lillehoj HS, Lillehoj EP.** Avian coccidiosis. A review of acquired intestinal immunity and vaccination strategies. Avian Dis 2000, **44**, 408-425.
87. **Lillehoj HS, Choi KD, Jenkins MC, Vakharia VN, Song KD, Han JY, Lillehoj EP.** A recombinant *Eimeria* protein inducing interferon-γ production. Comparison of different gene expression systems and immunization strategies for vaccination against coccidiosis. Avian Dis 200, **44**, 379-389.
88. **Lillehoj HS, Lillehoj EP, Yun CH.** Vaccine for avian enteropathogens *Eimeria*, *Cryptosporidium* and *Salmonella*. Animal Health Research Reviews 2000, **1**, 47-65.
89. **Lillehoj HS, Min W, Choi KD, Babu U, Burnside J, Miyamoto T, Rosenthal BM, Lillehoj EP.** Molecular, cellular and functional characterization of chicken cytokines homologous to mammalian IL-15 and IL-2. Vet Immunol Immunopathol 2001. **82**, 229-244.
90. **Lowenthal JW, York JJ, O'Neil TE, Rhodes S, Prowse SJ, Strom DG, Digby MR.** *In vivo* effects of chicken interferon-gamma during infection with *Eimeria*. J Interferon Cytokine Res 1997, **17**, 551-558.
91. **Lowenthal JW, O'Neil TE, Broadway M, Strom AD, Digby MR, Andrew M, York JJ.** Coadministration of IFN-gamma enhances antibody responses in chickens. J Interferon Cytokine Res 1998, **18**, 617-622.
92. **Lynch M, Walsh B.** Genetics and analysis of quantitative traits. pp. 502-503. Sinauer Associates, Inc. Publishers, Sunderland, 1998.
93. **Manly KF, Olson JM.** Overview of QTL mapping software and introduction to Map Manager QT. Mammalian Genome 1999, **10,** 327-334.
94. **Mayer L**. Local and systemic regulation of mucosal immunity. Aliment Pharmacol Ther 1997, **11**(Suppl. 3), 81-88.
95. **Mayer L.** Mucosal immunity and gastrointestinal antigen processing. J Pediatr Gastroenterol Nutr 2000,**30** (Suppl.), S4-12.
96. **Mckenzie SE, Mansfield E, Rappaport E, Surrey S, Fortina P**. Parallel molecular genetic analysis. Eur J Hum Genet 1998, **6**, 417-429.
97. **Michalski WP, Prowse SJ**. Superoxide dismutases in *Eimeria tenella*. Mol Biochem Parasitol 1991, **47**, 189-196.
98. **Min W, Kim J-K, Lillehoj HS, Sohn EJ, Han JY, Song KD, Lillehoj EP.** Characterization of recombinant scFv antibody reactive with an apical antigen of *Eimeria acervulina*. Biotechnol Letters 2001, **23**, 949-955.
99. **Murray HW, Rubin B, Carriero SM, Harris AM, Jaffe EA.** Human mononuclear phagocyte antiprotozoal mechanisms: oxygen-dependent and oxygen-independent activity against intracellular *Toxoplasma gondii*. J Immunol 1985, **134**, 1982-1988.
100. **Nakashima O, Terada Y, Inoshita S, Kuwahara M, Sasaki S, Marumo F.** Inducible nitric oxide synthase can be induced in the absence of active nuclear factor kappa B in rat mesangial cells: Involvement of the Janus kinase 2 signaling pathway. J Am Soc Nephrol 1999, **10**, 721-729.
101. **Rahman I, Macnee W.** Regulation of reduced glutathione levels and gene transcription in lung inflammation: therapeutic approaches. Free Radic Biol Med 2000, **28**, 1405-1420.
102. **Richards MP, Augustine PC.** Serum and liver zinc, copper, and iron in chicks infected with *Eimeria acervulina* or *Eimeria tenella*. Biol Trace Elem Res 1988, **17**, 207-219.
103. **Richards MP, Ashwell CM, Mcmurtry JP.** Analysis of leptin gene expression in chickens using reverse transcription polymerase chain reaction and capillary electrophoresis with laser-induced fluorescence detection. J Chromatogr 1999, **853**, 321-335.
104. **Richards MP, Ashwell CM, Mcmurtry JP.** Quantitative analysis of leptin mRNA using competitive reverse transcription polymerase chain reaction and capillary electrophoresis with laser-induced fluorescence detection. Electrophoresis 2000, **21**, 792-798.
105. **Richards MP, Caperna TJ, Elsasser TH, Ashwell CM, Mcmurtry JP.** Design and application of a polyclonal peptide antiserum for the universal detection of leptin protein. J Biochem Biophys Methods 2000, **45**, 147-156.
106. **Ruff MD.** Malabsorption from the intestine of birds with coccidiosis. In: Long P, Boorman K, Freeman B. (eds.), Avian Coccidiosis, pp. 281-295. British Poultry Sciences Ltd., Edinburgh, 1978.
107. **Sanderson IR, Walker Y.** Nucleotide uptake and metabolism by intestinal epithelial cells. J Nutr 1994, **124** (Suppl 1), 131S-137S.
108. **Sasai K, Lillehoj HS, Matsuda H, Wergin WP.** Characterization of a chicken monoclonal antibody that recognizes the apical complexes of *Eimeria acervulina* sporozoites and partially inhibits sporozoite invasion of CD8 lymphocytes *in vitro*. J Parasitol 1996, **82**, 82-87.
109. **Schmatz DM, Baginsky WF, Turner MJ.** Evidence for and characterization of a manitol cycle in *Eimeria tenella*. Molec Biochem Parasitol 1989, **32**, 263-270.

110. **Suzuki YJ, Ford GD.** Redox regulation of signal transduction in cardiac and smooth muscle. J Mol Cell Cardiol 1999, **31**, 345-353.
111. **Tanaka H, Makino Y, Okamoto K, Lida T, Yoshikawa N, Miura T.** Redox regulation of the nuclear receptor. Oncology 2000, **59** (Suppl.1), 13-18.
112. **Tirunagaru V, Sofer L, Burnside J.** An expressed sequence tag database of activated chicken T cells: Sequence analysis of 5000 cDNA clones. Genomics 2000, **66**, 144-151.
113. **Tomely F.** Antigenic diversity of the asexual developmental stages of *Eimeria tenella*. Parasite Immunol 1994, **6**, 407-413.
114. **Tomely F.** Techniques for isolation and characterization of apical organelles from *Eimeria tenella* sporozoites. Methods 1997, **13**, 171-176.
115. **Trees AJ, Crozier SJ, Mckellar SB, Wachira TM.** Class-specific circulating antibodies in infections with *Eimeria tenella*. Vet Parasitol 1985, **18**, 349-357.
116. **Trout JM, Lillehoj HS.** *Eimeria acervulina* infection: evidence for the involvement of CD8+ T lymphocytes in sporozoite transport and host protection. Poult Sci 1995, **74**, 1117-1125.
117. **Trout JM, Lillehoj HS.** T lymphocyte roles during *Eimeria acervulina* and *Eimeria tenella* infections. Vet Immunol Immunopathol 1996, **53**, 163-172.
118. **Uchida T, Kikuchi K, Takano H, Ogimoto K, Khaki Y.** Monoclonal antibodies inhibiting invasion of cultured cells by *Eimeria tenella*. J Vet Med Sci 1997, **59**, 721-723.
119. **Vainio O, Lassila O.** Chicken T cells: Differentiation antigens and cell-cell interactions. Crit Rev Poult Bio 1989, **2**, 97-102.
120. **Vallejo RL, Bacon LD, Witter RL, Cheng HH.** Genetic mapping of quantitative trait loci affecting susceptibility to Marek's disease in the chicken. In: Current Research on Mareks Disease. Proceedings of the 5th International Symposium on Marek's Disease. 1996.
121. **Vallejo RL, Bacon LD, Liu HC, Witter RL, Groenen MAM, Hillel J, Cheng HH.** Genetic mapping of quantitative trait loci affecting susceptibility to Marek's disease virus induced tumors in F2 intercross chickens. Genetics 1997, **148**, 349-360.
122. **van Kaam JBCHM, Arendonk JAM, Groenen MAM, Bovenhuis H, Vereijken ALJ, Crooijmans RPMA, van der Poel JJ, Veenendaal A.** Whole genome scan for quantitative trait loci affecting body weight in chickens using a three-generation design. Livest Prod Sci 1998, **54**, 133-150.
123. **van Kaam JBCHM, Groenen MAM, Bovenhuis H, Veenendaal A, Vereijken ALJ, van Arendonk JAM.** Whole genome scan in chickens for quantitative trait loci affecting growth and feed efficiency. Poult Sci 1999, **78**, 15-23.
124. **van Kaam JBCHM, Groenen MAM, Bovenhuis H, Veenendaal A, Vereijken ALJ, van Arendonk JAM.** Whole genome scan in chickens for quantitative trait loci affecting carcass traits. Poult Sci 1999, **78**, 1091-1099
125. **Xu S, Yonash N, Vallejo RL, Cheng HH.** Mapping quantitative trait loci for binary traits using a heterogeneous residual variance model: an application to Marek's disease susceptibility in chickens. Genetica 1998, **104**, 171-178.
126. **Xu-Amano J, Beagley KW, Mega J, Fujihashi K, Kiyono H, Mcghee JR.** Induction of T helper cells and cytokines for mucosal IgA responses. Adv Exp Med Biol 1992, **327**, 107-117.
127. **Yonash N, Bacon LD, Witter RL, Cheng HH.** High resolution mapping and identification of new quantitative trait loci (QTL) affecting susceptibility to Marek,s disease. Anim Genet 1999, **30**, 126-135.
128. **Yun CH, Lillehoj HS, Lillehoj EP.** Intestinal immune responses to coccidiosis. Dev Comp Immunol 2000, **24**, 303-324.
129. **Yun CH, Lillehoj HS, Choi KD.** Chicken IFN-γ monoclonal antibodies and their application in enzyme-linked immunosorbent assay. Vet Immunol Immunopathol 2000, **73**, 297-308.
130. **Yun CH, Lillehoj HS, Zhu J, Min W.** Kinetic differences in intestinal and systemic interferon-γ and antigen-specific antibodies in chickens infected with *Eimeria maxima*. Avian Dis 2000, **44**, 305-312.
131. **Yun CH, Lillehoj HS, Choi KD.** *Eimeria tenella* infection induces local IFN-γ production and intestinal lymphocyte subpopulation changes. Infect Immun 2000, **68**, 1282-1288.
132. **Zhang S, Lillehoj HS, Ruff MD.** *In vivo* role of tumor necrosis-like factor in *Eimeria tenella* infection. Avian Dis 1995, **39**, 859-866.
133. **Zhang S, Lillehoj HS, Ruff MD.** Chicken tumor necrosis-like factor. I. *In vitro* production by macrophages stimulated with *Eimeria tenella* or bacterial lipopolysaccharide. Poult Sci 1995, **74**, 1304-1310.
134. **Zhou Y-X, Kalocsai P, Chen J-Y, Shams S.** Information processing issues and solutions associated with microarray technology. In: Schena M. (ed.), Microarray Biochip Technology. pp. 167-200. BioTechnique, Natick, 2000.