Immune response to Coccidia

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SUMMARY

In view of rapid development of new biotechnologies in veterinary science, novel control strategies using genomics, molecular biology and immunology will offer an alternative way to prevent the spread of coccidiosis in the future. Increasing understanding of the protective role of local intestinal immune responses and the identification of various effector molecules against coccidia provide optimism that novel means to control coccidiosis will be feasible in the near future. The aim of this presentation is to review the current progress in our understanding of the host immune response to Eimeria and to discuss potential strategies which are currently being developed for coccidiosis control. Due to complexities of the host immune system and the parasite life cycle, comprehensive understanding of host-parasite interactions that lead to protective immunity should precede before we can develop successful prevention and disease control strategies. Recent progress in Eimeria and poultry genome sequencing and rapidly developing functional genomics technology is facilitating the identification and characterization of host and parasite genes which are involved in immunoprotection and immunopathology in avian coccidiosis. Recent studies have provided much evidence that molecular and immunological-based strategies such as recombinant vaccines and dietary immunomodulation enhance gut immunity. Thus, successful application of new knowledge on host-parasite immunobiology, gut immunity and genomics in commercial settings will lead to the development of novel disease prevention strategies against coccidiosis in the near future.

INTRODUCTION

Avian coccidiosis is the major parasitic disease of poultry with a substantial economic burden estimated to cost the industry greater than $800 million in annual losses (Williams, 1998). In-feed medication for prevention and treatment contributes a major portion of these losses in addition to mortality, malabsorption, inefficient feed utilization and impaired growth rate in broilers, and a temporary reduction of egg production in layers. Eimeria spp. possess a complex life cycle comprising of both sexual and asexual stages, and their pathogenicity varies in birds of different genetic backgrounds (Lillehoj, 1988). In the natural host, immunity is species-specific, such that chickens immune to one species of Eimeria are susceptible to others. Additionally, Eimeria spp. exhibits different tissue and organ specificity in the infected host. Understanding the interplay between the host and the parasites in the intestine is crucial for the design of novel control approaches against coccidiosis.

While natural infection with Eimeria spp. induces immunity, vaccination procedures on a commercial scale have shown limited effectiveness and current disease control remains largely dependent on routine use of anti-coccidial drugs in most countries (Dalloul and Lillehoj, 2005). Recently several different live vaccines have been commercially developed and these are mostly composed of either virulent or attenuated parasite strains. Major disadvantages of live parasite vaccines are labor-intensive production and high cost due to inclusion of multiple parasite species in the vaccine. Although live oocyst vaccines represent a limited but useful alternative to anticoccidial drugs, a recombinant vaccine composed of parasite antigens/antigen-encoding genes that elicit coccidia-specific immunity would be eminently preferable. While it would be cost-effective to produce recombinant vaccines (proteins or DNA), the difficulty remains to identify the antigens or genes which are responsible for eliciting protective immunity and to devise the most efficient delivery method for these recombinant vaccines to be delivered and presented to the bird's immune system. Also, such subunit vaccines would eliminate the danger of emerging resistant strains encountered with live vaccines, but unfortunately until efficient vaccines become commercially available, the poultry industry is forced to rely upon prophylactic chemotherapy to control coccidiosis. Further, the introduction of alternative prevention/treatment measures such as non-chemical feed supplements

that effectively enhance productivity and non-specific immunity may help limit the use of anticoccidials. However, the lack of efficient vaccines and the increasing incidence of drug resistant strains and escalating public anxiety over chemical residues in meat and eggs mandate the development of alternative control methods. For developing a successful recombinant vaccine strategy, we need to better understand the chicken immune system and the means to elicit effective immunity against coccidia.

PATHOGENESIS AND IMMUNOGENICITY OF Eimeria

The pathogenicity of coccidia depends largely on the successful replication of developing parasites inside the host. Theoretical estimates indicate that a single oocyst of a virulent species such as E. tenella could yield 2,520,000 invasive parasites after the 2nd merogony stage (Levine, 1982). E. maxima is thought to undergo a minimum of four generations of schizogony (McDonald et al., 1986). Most major enteric protozoa including coccidia invade the intestinal mucosa and induce a certain degree of epithelial cell damage, inflammation and villous atrophy (Pout, 1967). The signs of coccidiosis depend on the degree of the damage and inflammation and include watery, whitish diarrhea (E. acervulina) or hemorrhagic diarrhea (E. tenella), petechial hemorrhages and the marked production of mucus (E. maxima), dehydration, weight loss, rectal prolapse and dysentery. The profuse bleeding in the ceca is a characteristic feature of E. tenella infection due to its extensive destruction of the mucosa with histological lesions (Witlock et al., 1975).

In general, young animals are more susceptible to coccidiosis and more readily display signs of disease, whereas older chickens are relatively resistant to infection (Lillehoj, 1998). Young animals which recover from coccidiosis may later be able to partly compensate for the loss of body growth, but their growth potential remains severely compromised. The magnitude of clinical signs resulting from Eimeria infection is significantly influenced by host genetic factors. In two genetically divergent strains of inbred chicken lines, SC (B2B2) and TK (B15B21), the different degrees of disease pathogenesis depended upon the genetics of host when they are infected with E. tenella or E. acervulina (Lillehoj, 1998). In general, SC chickens are more resistant than TK chickens to coccidiosis. Because of their genetic differences in disease susceptibility to coccidiosis, extensive research has been carried out regarding the underlying immunological mechanisms controlling intestinal cell-mediated immune responses. More detailed information concerning the genetic control of immune response to Eimeria can be found in previous reviews (Lillehoj, 1991 and 1998; Lillehoj and Lillehoj, 2000, Lillehoj et al., 2004).

Infection with Eimeria induces protective immunity that is long-lasting and exquisitely specific to that particular Eimeria species. While a large number of oocysts is generally required to generate a good immune response against Eimeria, some exceptions have been noted, e.g. E. maxima is highly immunogenic and requires only a small number of oocysts to induce almost complete immunity. The early endogenous stages of Eimeria life cycle are considered to be more immunogenic than the later sexual stages (Rose and Hesketh, 1976; Rose et al., 1984) although gamete antigens of E. maxima were shown to be immunogenic and induce protection against a challenge infection with the live parasites (Wallach et al. 1990 and 1995). However, activation of immune T cells released IFN-g which inhibited the intracellular development of coccidia (Lillehoj and Choi, 1998) indicating that host protective immunity is against the exponential growth phase of the parasite life cycle. Because of the complexity of host-parasite immunobiology which involve many different cell types and soluble factors, further studies are necessary to obtain insights on host immunity eliciting complete protection.

CHICKEN IMMUNE SYSTEM

Chickens have evolved sophisticated immune system much like mammals. Major defense mechanisms include a non-specific immune response which is activated immediately following exposure to potential pathogens. Non-specific immunity is mediated by macrophages, granulocytes, natural killer (NK) cells, serum proteins and soluble factors and precedes the development of antigen-specific memory immune response mediated by lymphocytes. Lymphocytes are generated in the primary lymphoid organs such as thymus and bursa of Fabricius where they acquire functional identity whereas it is in the secondary lymphoid organs such as lymph nodes, spleen and mucosal associated lymphoid tissues where they differentiate into effector cells upon encounter with antigens and potential pathogens. Lymphoid organs are organized into different compartments where lymphocytes and non-lymphoid cells form a microenvironment suitable for effective immune responses. In summary the major cellular components of the avian immune system include thymus-derived T lymphocytes, bursa-derived B lymphocytes, macrophages and NK cells.

B lymphocytes: B lymphocytes play an important role in host defense against many infectious diseases by producing antibodies which are specific for the eliciting antigen. Once produced, antibodies becomes effector molecules which can either block the invasion of host cells by pathogens, neutralize toxins or kill extracellular pathogens through antibody-dependent cell-mediated cytotoxicity (ADCC). Unlike other animals, chicken B cells develop in the bursa of Fabricius, a gut associated primary lymphoid tissue located near the cloaca. During embryonic development, pre-bursal stem cells enter

the bursal rudiment in a single wave (Houssaint et al., 1976) where they undergo a maturation process which involves generation of antibody diversity by gene conversion and somatic diversification to generate different classes of immunoglobulins (IgM, IgG and IgA.) Bursal cells migrate out of bursa to the periphery a few days prior to hatch. Chickens generate strong antibody responses to both T cell-dependent and independent antigens. After an initial encounter with an antigen, B cells secrete the IgM isotype of antibody which later switches to IgG or IgA upon the secondary exposure. As in mammals, the secondary response is characterized not only by isotype switching but also an increase in magnitude compared with the initial response (Ratcliff, 1989). The activation of naٸve B cells in vivo requires a direct interaction with helper T cells typically expressing CD4 and this interaction is restricted by antigen recognition in the context of class II genes of the major histocompatibility complex (MHC).

T lymphocytes: Thymus-derived lymphocytes in chickens are divided into 3 separate subpopulations on the basis of their cell surface antigen expression and biological function. Unlike mammals that possess two different types of antigen-recognizing receptors (TCRab and TCRgd), chicken T cells express 3 distinct T cell receptors, TCR1, TCR2 and TCR3. The TCR on a given T lymphocyte subset can be a heterodimer consisting either of a g and d chain (TCR1), an a and Vb1 chain (TCR2) or an a and Vb2 chain (TCR3) (Cooper et al., 1991; Gobel, 1996). As in mammals, immature T lymphocytes undergo differentiation in the thymus in chickens: CD4-CD8- thymocytes give rise to CD4+CD8+ which develop into CD4+CD8- or CD4-CD8+ T-cells. CD4 and CD8 T subsets express all 3 types of TCRs (Davidson and Boyd, 1992). Mature CD4 or CD8 single-positive T-cells leave the thymus to populate secondary immune organs, and also travel in the circulatory and lymphatic systems. In the blood, CD4-CD8-TCR1+ T cells, as well as CD4+CD8- and CD4-CD8+ T cells, expressing either TCR2 or TCR3 have been identified. The same T cell populations are found in the spleen where CD4-CD8+TCR1+ T cells also exist (Sowder et al., 1988; Gobel, 1996). With differentiation, functionally distinct T cell subsets express certain cell surface proteins. T cells expressing both CD4 and CD8 molecules are considered immature T cells and constitute the majority of cells in the thymus. Single-positive T cells, expressing either CD4 or CD8 are mature T cells. Most CD4+CD8- cells are helper or inflammatory T cells responding to exogenous antigen in association with MHC class II molecules, whereas CD4-CD8+ cells represent cytotoxic cells which respond to endogenous antigen in association with MHC class I molecules. The ab TCR (TCR2 and TCR3) are known to mediate MHC-restricted antigen recognition by single-positive T cells, whereas the physiological role of T cells expressing TCR1 is not well defined (Gobel, 1996).

Macrophages: Macrophages and dendritic cells represent components of the mononuclear phagocyte system (van Furth et al., 1972) and are involved in processing and presenting antigens to lymphocytes. Macrophages are highly heterogeneous cells present in primary and secondary lymphoid tissues and are important cells involved in host defense. Their functions are primarily phagocytosis, cytotoxic activity against tumors and production of chemokines and cytokines which mediate inflammatory responses. In chickens, interdigitating cells (IDC) which are found in situ in all T cell areas of all lymphoid tissues are characterized by dendritic extensions and express high level of MHC class II antigens and are probably the main antigen presenting cells to T helper cells in vivo during primary immune responses (Jeurissen et al., 1994). Macrophages express many cell surface antigens that have been detected by mouse monoclonal antibodies including K1 (Lillehoj et al., 1993) and KUL01 (Mast and Goddeeris,1995). Recent advances in our understanding of how these cells recognize diverse antigens led to the discovery of highly conserved pattern recognition receptors (PRRs) which are involved in recognition of highly conserved molecular structures on microbial components called pathogen-associated molecular patterns (PAMPs). The Toll-like receptor (TLR) family is membrane-bound PRRs that play critical roles in activating the innate immune response and phagocytosis (Underhill and Ozinsky, 2002). Activation of TLR by binding to a particular ligand leads to activation of the NF-kB signal transduction pathway inducing a wide variety of host genes involved in innate immunity, such as antimicrobial peptides, cytokines, chemokines and nitric oxide synthase (Barton and Medzhitov, 2002). Although in humans more than ten TLRs have been identified, only a limited number of homologues have been characterized in chickens. Thus, it appears that TLRs have been conserved through evolution and expressed in various immune related tissues and cell lines (Iqbal et al., 2005). Similar to the mammalian gene products, the secondary protein structure of chicken TLR1, 3, 5, 7, and 10 consist of several leucine rich domains, a transmembrane domain, and Toll/interleukin-1 receptor domains (Yilmaz et al., 2005). Understanding of how TLRs regulate immune response to pathogens in poultry will be important for future development of new strategies for disease control.

NK cells: NK cells are non-lymphoid, heterogeneous and nonphagocytic cells which mediate immediate response against infection. Along with macrophages, they are important in defense against pathogens and tumors in unimmunized hosts. NK cells in chickens have been identified from freshly obtained spleen of SPF chickens (Schat et al., 1986) and have been identified in many tissues including blood and the intestine (Lillehoj and Chai, 1988). In mammals, NK cells express the ? chain of CD3, the IL-2 receptor, CD2, and CD16. Chicken NK cells have been identified using different monoclonal antibodies such as K108 (Chung and Lillehoj, 1991) and 28-4 and they do not express CD3, CD4 or TCR (Gobel et al. 1996). Rather, NK cells have been classified as TCR0 cells since they do not express TCRab or TCRgd. NK cells have been implicated in resistance against MDV-induced tumors (Lam and Linna, 1979), and in intestinal defense against coccidia (Lillehoj, 1989) and rotavirus (Myers and Schat, 1990).

INNATE AND AQUIRED IMMUNE RESPONSE TO Eimeria

Because the life cycle of Eimeria parasites is complex and comprised of intracellular, extracellular, asexual, and sexual stages, host immune responses are quite diverse and complex. After invasion of the host intestine, Eimeria elicit both nonspecific and specific immune responses which involve many facets of cellular and humoral immunity (Lillehoj, 1991; Lillehoj 1998; Lillehoj and Lillehoj, 2000; Dalloul and Lillehoj, 2005) Nonspecific factors include physical barriers, phagocytes, leukocytes, chemokines and complement components. Antigen-specific immunity is mediated by antibodies, lymphocytes, and cytokines. Due to the specific invasion and intracellular development of coccidia in the intestine, understanding of the gut-associated lymphoid tissues (GALT) is important. The GALT serve three main functions in host defense against enteric pathogens: processing and presentation of antigens, production of intestinal antibodies, and activation of cell-mediated immunity (CMI). In the naıve host, coccidia activate local dendritic cells and macrophages eliciting various chemokines and cytokines (Lillehoj, 1998). In immune hosts, parasites enter the gut early after infection, but are prevented from further development, indicating that acquired immunity to coccidiosis may involve mechanisms that inhibit the natural progression of parasite development (Rose et al., 1984; Trout and Lillehoj, 1996; Lillehoj and Choi, 1998; Yun et al., 2000a). Recent studies demonstrated the role of several cytokines produced locally during coccidiosis (Yun et al., 2000b; Min et al., 2003; Lillehoj et al., 2004; Dalloul and Lillehoj, 2005) which are responsible for enhancing protective immunity against Eimeria (Lillehoj et al., 1998; Yun et al., 2000c).

Antibody responses: Following coccidiosis, both circulating and secretory antibodies specific for coccidia parasites are detected in serum, bile and intestine (Lillehoj and Ruff, 1987; Lillehoj, 1988; Yun et al., 2000c). However, antibody titers in serum and intestine do not correlate with the level of protection after oral infection with coccidia (Dalloul et al., 20003; Lillehoj and Ruff, 1987). Convincing evidence on the minimum involvement of humoral antibody to limit coccidian infection came from agammaglobulinemic chicken models where it was observed that chickens Bursectomized by hormonal and chemical means were resistant to reinfection with coccidia (Rose and Long, 1970; Lillehoj, 1987). Three isotypes of antibodies are recognized in birds, IgM, IgA, and IgY. IgY is considered the orthologue of the mammalian IgG (Leslie at al., 1969), even though the cDNA encoding the IgY heavy chain shows similarity to mammalian IgE (Parvari et al., 1998). The presence of other antibody classes such as IgD or IgE in chickens has not yet been documented. The role of parasite specific antibodies both in serum and mucosal secretions has been extensively studied in coccidiosis (Girard et al., 1997; Lillehoj and Ruff, 1987). Maternal IgY is concentrated in the yolk sac of the egg (Rose et al., 1974) where it is transported to the embryo during late development by a mechanism similar to that found in mammals (West el al., 2004), and is thus considered to be of some relevance in maternal passive immunity (Wallach et al., 1992). When hens were hyperimmunized with gametocyte surface antigens of E. maxima, passively transferred antibodies in young birds protected against challenge with sporulated E. maxima oocysts by reducing fecal oocyst production (Wallach et al., 1992). The efficiency of maternally transferred antibodies in protection against field infections needs to be verified using large scale trials. Considering the short life span of maternal antibodies in young chicks, it may not be feasible to maintain extremely high levels of antibodies in birds for a long time.

Lately, immunotherapy using whole antibody molecules or single chain fragments of the variable region (ScFv) with antigen binding activity has been gaining interest as a potential immunotherapy against infectious agents. Currently available immunological control strategies consist of sub-acute infection with virulent or live attenuated parasites. The main obstacle to the development of an antibody-based strategy against avian coccidiosis however, is the existence of many different Eimeria species. With recent progress in molecular biology and sequence information on chicken immunoglobulin genes, it is now possible to generate recombinant chicken antibodies (Min et al., 2001; Park et al., 2004). There are potentially two different approaches using antibodies against coccidiosis. One is to produce hyperimmune serum against major immunogenic proteins of coccidia and passively administer it to 18 day-old embryos or to feed orally to young chicks at hatch. In a previous study, a surface protein 3-1E, which was identified from the merozoites of E. acervulina, was used as a potential subunit vaccine for avian coccidiosis and found to be protective against challenge infection with the homologous Eimeria (Lillehoj et al., 2000). Moreover, a DNA vaccine prepared from the gene coding for the protein was partially protective against challenge with E. acervulina (Lillehoj et al., 2000). These results prompted us to investigate the potential use of chicken antibodies against 3-1E in protection against coccidiosis. In a recent report (Ngyen et al., 2003), we tested the protective effect of chicken egg antibody (IgY) powder which was prepared from hens hyperimmunized with purified 3-1E recombinant protein in a challenge model with E. acervulina and E. tenella. Chickens which were fed standard diet with IgY powder containing antibodies against 3-1E (3-1E/IgY) were better protected against oral challenge with E. tenella or E. acervulina oocysts compared with those fed with standard diet supplemented with IgY-containing powder only. These results clearly indicated that 3-1E represents an important target antigen for coccidiosis prevention and that passive immunization of chickens with antigen-specific IgY powder is a promising method to confer protection against coccidiosis.

Another approach to generate therapeutic antibody is to develop recombinant antibodies against protective epitopes. Using the chicken B cell line R27H4, we previously developed several hybridomas producing coccidia-specific antibodies

(Lillehoj et al., 1994). One of them, 6D-12-G10, was reactive with an Eimeria protein suggested to be involved in binding to a host cell receptor (Sasai et al. 1996). Unfortunately, the amount of antibody secreted by this hybridoma into culture medium was insufficient for further biochemical and physiological characterization of the antigen. To circumvent the problems associated with low yield, we produced an scFv fragment derived from the VH and VL genes encoding the 6D-12-G10 antibody. The single chain Fv antibody was expressed in E. coli and the recombinant gene product bound whole parasites (Min et al., 2002) by immunoblot, immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA). Chickens fed recombinant scFv antibodies showed reduced fecal oocysts upon challenge infection with live coccidia (unpublished data). Using similar approaches, we also generated other scFv antibodies detecting coccidia proteins (Park et al., 2004). Like the native monoclonal antibodies from which they were derived, these recombinant antibodies showed binding activity against Eimeria antigens and were secreted at 5 mg/L into culture medium, indicating that soluble, stable and functional chicken ScFv can be produced in large volume. Thus, recombinant antibody technology has advantages over hybridomas, which generally produce low quantities of antibodies (<0.5 mg/L), easily lose antibody activity and are not able to make high titer ascites.

Although the role of antibodies produced during natural infection is debatable, antibodies which are generated against specific epitope of coccidian parasites can be used to reduce parasite invasion and have been shown to be beneficial against coccidiosis infection (Walach et al., 1992; Ngyen et al., 2003). Antibody-based therapies can be useful, for example, to prevent Eimeria infections, where antibodies are known to play a role in protection against the parasite. The results presented here demonstrate an example of recombinant chicken antibodies useful to reduce parasites in the field. ScFv antibody fragments may offer advantages for in vivo applications as diagnostic and therapeutic reagents also. For example, because the scFv antibody is approximately 33 kiloDalton in size, representing 20% of an intact IgG molecule, it may penetrate tissues easily, an important consideration given the invasive nature of Eimeria parasites. The ability to generate unlimited amount of soluble and functional recombinant scFv antibodies will facilitate the investigation of their potential therapeutic value in passive immunotherapy against avian coccidiosis. Meanwhile evidence that antibodies in dietary supplements could protect against oral coccidiosis infection opens a new door for novel immunotherapy strategies against coccidiosis. Furthermore, given the limited information concerning the nature of protective antigens of Eimeria, these antibodies will be an important tool for affinity isolation of potential Eimeria subunit vaccines.

Cell-mediated immunity: The evidence that the removal of the bursa by chemical or hormonal means (Rose and Long, 1970; Lillehoj, 1987) did not interfere with the development of protective immunity against Eimeria indicated the importance of cell-mediated immunity in coccidiosis. The role of T cells in the protection against coccidiosis has also been studied in immunosuppressed chickens using T cell-specific drugs that selectively abrogate or severely impair T cell function. These treatments included thymectomy (Rose and Long, 1970), cyclosporin A (Lillehoj, 1987), betamethasone, dexamethasone (Isobe and Lillehoj, 1993), and cell depletion using mouse monoclonal antibodies against CD8+ or abTCR-expressing cells (Trout and Lillehoj, 1996). In all of these studies, the abrogation of T cell function impaired host protective immunity against coccidiosis. Additional evidence for the protective role of T cells came from adoptive transfer studies where peripheral blood lymphocytes (PBL) and spleen cells from E. maxima-immune chickens protected syngeneic recipients against a live parasite challenge infection (Rose and Hesketh, 1982). Lillehoj and Choi (1998) and Miller et al. (1994), using an in vitro culture, showed that splenocytes from E. tenella-immune chickens inhibited the intracellular development of E. tenella in kidney cells. The nature of these cells was not determined, but may be NK cells since they did not show any MHC restriction in their action. Direct evidence for the presence of Eimeria-specific T cells was demonstrated by an in vitro antigen-driven lymphoproliferation assay (Rose and Hesketh, 1984; Lillehoj, 1986; Vervelde et al.,1996).

T lymphocytes: In the gut, intraepithelial lymphocytes (IEL) represent an important component of the GALT (Guy-Grand et al., 1974). A unique feature of IEL is that gd T cells are predominant, whereas the vast majority of mature T lymphocytes in the peripheral blood and lymphoid organs use the CD3-associated ab TCR heterodimer for antigen recognition (Goodman and Lefrancois, 1988; Bonneville et al., 1988). Following primary and secondary infections with E. acervulina, an increased percentage of intraepithelial gd T cells was observed in the duodenum (Choi and Lillehoj, 2000). The percentage of gd T cells was significantly elevated by day 8 following primary infection with E. acervulina in SC chicken whereas a significant increase was seen as early as day 4 in TK chickens (Choi et al., 1999). Concurrent with the increase of gd T cells, a significant enhancement of IL-2 mRNA transcripts was found (Choi and Lillehoj, 2000). The percentage of ab T cells was elevated in IEL by day 4 after primary infection with E. acervulina in SC chickens whereas a significant increase of ab T cells was not seen until 6 d post secondary infection in SC chickens (Choi et al., 1999).

The importance of CD8+ T cells has been shown in many intracellular parasitic infections including toxoplasmosis (Hakim et al., 1991) and malaria (Weiss et al., 1990). In avian coccidiosis, the selective elimination of CD8+ cells by anti-CD8+ monoclonal antibody resulted in exacerbation of the disease, as evidenced by increased oocyst shedding after infection with E. tenella or E. acervulina (Trout and Lillehoj, 1996). Significant increase of T cells expressing CD8+ molecules was noted in the intestinal IEL population following challenge infections of chicken with E. acervulina (Lillehoj and Bacon,

1991). Two-color immunofluorescence staining revealed that the majority of CD8+ cells in the duodenum intraepithelium of immune chickens co-expressed the ab TCR. In both SC and TK chickens, the ratio of CD8+ to CD4+ T lymphocytes in IEL was elevated by day 4 following primary and secondary infections with E. acervulina. These cells continued to increase in SC chickens but showed a marked decrease in TK chickens following the secondary infection (Choi et al., 1999). When two MHC congenic chickens with a different disease susceptibility to coccidiosis were compared, the higher increase of ab TCR+CD8+ and gd TCR+CD8+ cells was associated with B2B2 chickens which are less susceptible. Similarly, Bessay et al. (1996) observed a significant increase in the proportion of CD4+, CD8+ and TCR gd cells in duodenal IEL from day 4 to day 8 post-infection with E. acervulina. In contrast, the proportion of CD8+ cells decreased significantly in the blood and spleen on days 4 and 6 post-infection. After E. tenella infection, the proportion of CD4+ cells increased on day 8 post-infection and CD8+ cells on days 6 and 8 post-infection in cecal IEL. At the same time, the proportion of CD4+ cells decreased in the spleen on day 8 post-infection and CD8+ cells decreased in the blood on day 6. In chickens infected with E. mivati, the percentages of splenic lymphocytes bearing CD8+, gd TCR, class II MHC, or surface IgM antigens, were decreased in the dexamethasone-treated chickens when compared to the normal chickens (Isobe and Lillehoj, 1993). Significantly higher numbers of total oocyst output in the dexamethasone-treated chickens following primary and secondary infections with E. mivati indicated the significance of CD8+ cells in primary as well as secondary immune responses.

In the peripheral blood, a transient but sharp increase in the proportion of CD8-expressing T cells was found in White Leghorn chickens at 8 days after a primary infection with E. tenella (Breed et al., 1996; 1997a,b). This increase was found to be concurrent with a marked increase in IFN-g as well as nitric oxide (NO) production upon in vitro stimulation of PBL by T cell mitogen and E. tenella sporozoite antigen (Breed et al., 1997a). In E. maxima infection, both CD4+ and CD8+ cells were seen in the small intestine of Light Sussex chickens, but the proportion of CD8+ cells was higher (Rothwell et al., 1995). CD4+ cells represent a minor population of the IEL. During E. acervulina infection, CD4+ cells increased at day 7 after primary and day 14 after secondary infection (Lillehoj, 1994). Bessay et al. (1996) examined the T-lymphocyte subsets in the intestine following E. tenella and E. acervulina infections. Following E. acervulina infection, a significant increase in the proportion of CD4+ was observed in duodenal IEL from day 4 to day 8, and in the blood and spleen on day 8 post-infection. In E. tenella infection, CD4+ cells increased on day 8 post-infection in the cecal IEL but the proportion of CD4+ cells dropped in the spleen on day 8 post-infection. In the ceca, the number of CD4+ cell increased significantly at day 2 after E. tenella infection and in immune chickens, mainly CD4+ and CD8+ T cells infiltrated the lamina propria (Vervelde et al., 1996). A significantly higher number of sporozoites were found within or next to CD3+, CD8+, and ab TCR+ cells in immune chickens. In a study aimed at elucidating the immunologic differences between resistant SC (B2B2) and susceptible TK (B15B21) chickens, duodenal CD4+ T lymphocytes increased significantly and rapidly at day 4 after primary and secondary infections with E. acervulina in SC as compared to TK chickens (Choi et al., 1999). The role of CD4+ T cells in coccidiosis may involve the production of soluble cytokines such as IFN-g (Yun et al., 2000a,b,c). Using a quantitative RT-PCR, increased IFN-g mRNA expression was observed in the cecal tonsil lymphocytes in E. tenella-infected SC chickens, and the selective depletion of CD4+ cells, but not CD8+ cells, reduced IFN-g production.

Non-T Cells:The role of NK-cells in parasitic diseases has been well documented (Lillehoj et al., 2004). The chicken gut IEL are known to contain subpopulations of cells that can mediate NK cell activities as demonstrated in 4 hr 51Cr release assays using different avian tumor cell targets (Chai and Lillehoj, 1988). The NK cell activity was higher in the jejunum and ileum than in the duodenum and cecum. Following infection with Eimeria parasites, the NK cell activities of both splenic and intestinal IEL decreased to a subnormal level during the early phase of infection (Lillehoj, 1989). NK cell activity returned to normal or slightly higher than normal levels about 1 week after the primary inoculation. Significant increases in the splenic and intestinal IEL NK cell activities were seen during the early phase of secondary infection. This increase in the IEL NK cell activity shortly after secondary infection was accompanied by a substantial increase in the number of IEL expressing the asialo-GM1 antigen, a NK marker (Lillehoj, 1989). In a recent study, we have identified a major effector molecule from IEL which shows lytic activity against sporozoites (unpublished observation). Chicken NK cells, defined phenotypically as CD8+ cells lacking T- or B lineage specific markers, constitute approximately 30% of CD8+ intestinal IEL, but < 1% of splenocytes or PBL (Gobel et al., 2001). Using the 28-4 monoclonal antibody, specific for CD8+CD3- IEL and an antibody for CD3, IEL were separated into CD3+ IEL T cells and the 28-4+ cells, both co-expressing the CD8 antigen. The 28-4+ IEL were able to lyse the NK-sensitive target cells. These results define the two major phenotypically and functionally distinct IEL subpopulations, and imply an important role of NK cells in the mucosal immune system (Gobel et al., 2001). Using mouse antibodies K-14 and K-108, Chung and Lillehoj (1991) identified NK cells which stain 6 to 17% of splenic lymphocytes, 11 to 14% of PBL, and fewer than 5% of thymic and bursal lymphocytes.

Chicken macrophages identified using the monoclonal antibody K1, express MHC class II antigens (Kaspers et al., 1993) and are involved in different phases of the host immune response to coccidia (Lillehoj et al., 2004). In E. tenella-immune chickens, more leukocytes were present in the lamina propria and leukocytes infiltrated the ceca more rapidly than in the naive chickens (Vervelde et al., 1996). By immunocytochemical staining, most infiltrating leukocytes were macrophages

and T cells. Macrophages pretreated with the culture supernatants of Con A-stimulated spleen cells or T cells exerted cytostatic effects on the growth of E. tenella sporozoites (Lillehoj et al., 1989; Dimier et al., 1998). Pretreatment of macrophages with culture supernatants of Con A-stimulated spleen cells induced NO synthesis, and the addition of NG monomethyl-L-arginine, a NO synthase inhibitor, also overcame the inhibition of E. tenella replication in macrophage cultures suggesting possible involvement of NO or toxic oxygen intermediates in inhibiting E. tenella growth (Dimier-Poisson et al., 1999).

Cytokine and chemokine responses: Extensive experimental evidence supports the notion that immunity mediated by lymphocytes and their secreted products such as cytokines mediate antigen specific protection against challenge infection with Eimeria (Lillehoj and Lillehoj, 2000; Lillehoj et al., 2004). For example, correlation of disease resistance and enhanced proliferation of T lymphocytes and recruitment of intestinal T cells into the duodenum following primary and secondary E. acervulina infections have been documented (Lillehoj, 1989 and 1998; Lillehoj et al., 2004). In the intestine, lymphocytes, macrophages, dendritic cells and other effector cells act in harmony to secrete cytokines and proinflammatory molecules which direct the appropriate immune responses to eliminate the invading parasite and to induce the development of memory responses. Recently, using T lymphocyte and macrophage cDNA microarrays, host genes related to the immune response in the gut have been identified and their role in protection against coccidiosis is being investigated (Min et al., 2003 and 2005; Dalloul et al., in preparation). Although the importance of cytokines in mediating innate and acquired immunity against coccidiosis has been suggested and documented, the nature of many chicken cytokines has not been well characterized due to slow progress in characterizing chicken cytokine genes (Lillehoj et al., 2003). In contrast to the plethora of mammalian cytokines, only a few chicken homologues have been described, the main ones being IFN-g, TGF, TNF, IL-1, IL-2, IL-6, IL-8 and IL-15 (Lillehoj, 2004). Of late, a series of new chicken cytokines have been described including IL-17 (Min and Lillehoj, 2002), IL-18 (Gobel et al., 2003), IL-16 (Min and Lillehoj, 2003), IL-12(Degen et al., 2004), and Th2 type cytokines such as IL-3, IL-4, IL-13 and GM-CSF (Avery et al., 2004), IL-10 (Rothwell et al., 2004) and IL-5 (Koskela et al., 2004).

Many different types of chemokines and cytokines are produced following primary and secondary infection with Eimeria (Lillehoj et al., 2003). Interferons have been shown to have various immunomodulating effects on a wide variety of tissues. Interferons are classified into type I (IFN-a,-b, -w, and -t) and type II (IFN-g). The chicken gene encoding IFN-g has been cloned and its biological function studied by many laboratories (Digby and Lowenthal, 1995; Song et al., 1997). IFN-g production during coccidiosis was examined using a quantitative RT-PCR (Choi et al., 1999; Yun et al., 2000c), and recently using gene expression profiling (Min et al., 2003). After E. acervulina infection, IFN-g mRNA expression was detected in the cecal tonsils and spleen but not in the duodenum of SC chickens (Choi et al., 1999). In E. tenella-infected chickens, IFN-g transcripts were detected in the spleens, cecal tonsils, and IEL following the primary and the secondary infections with E. tenella. The marked increase in the transcripts of IFN-g was shown at day 6 after primary infection in the cecal tonsils. Laurent et al. (2001) recently showed that IFN-g expression in the cecum and jejunum of White Leghorn (PA12) chickens increased over 200-fold above the control at day 7 after primary infection with E. tenella and E. maxima. The effects of pretreatment of chicken macrophages or fibroblasts with crude culture supernatants containing IFN-g on E. tenella sporozoites were examined in various in vitro systems (Lillehoj et al., 1989; Lillehoj and Choi, 1998; Dimier et al., 1998). Multiple intramuscular injections (three times) of the supernatant of recombinant chicken IFN-g at one day prior to, and two and four days after infection with E. acervulina,, conferred significant protection as measured by body weight loss and oocyst shedding in both SC and TK strains (Lillehoj and Choi, 1998). Furthermore, E. tenella sporozoites were inhibited to undergo intracellular development in a chicken cell line stably transfected with the chicken IFN-g gene. Treatment of chicken cells with recombinant IFN-g inhibited the intracellular development of E. tenella without affecting sporozoite invasion of host cells (Lillehoj and Choi, 1998). These results provide the first direct evidence that IFN-g exerts an inhibitory effect against Eimeria and provides a rational basis for the use of this cytokine as a vaccine adjuvant against coccidiosis.

Interleukin-2 (IL-2) is a potent growth factor for a variety of cell types including T cell differentiation, B cell development and NK cell activation (Lillehoj et al., 1992; Farner et al., 1997). The chicken IL-2 gene has been cloned (Sundick and Gill-Dixon, 1997) and its biological function characterized (Choi and Lillehoj, 2000; Lillehoj et al., 2001). After primary and secondary infections with E. acervulina, a significant enhancement of IL-2 mRNA transcripts was observed in the spleen and intestine (Choi and Lillehoj, 2000). The protective effect of IL-2 on vaccination of chickens with the recombinant 3-1E coccidia gene was recently demonstrated by DNA vaccination (Lillehoj et al., 2000; Min et al., 2001). Co-injection of the IL-2 gene with the 3-1E or MIC2 antigen or gene enhanced the host response to the vaccination procedure (Ding et al., 2005a,b; Lillehoj et al., 2004, 2005).

IL-16 was originally described as a lymphocyte chemoattractant factor synthesized by CD8+ and CD4+ T cells and released in response to antigens, mitogens, histamine or serotonin (Cruikshank et al., 2000). Further analysis indicated that IL-16 is generated by B cells, mast cells, epithelial cells, macrophages, fibroblasts, and eosinophils (Cruikshank et al., 2000). Initially, IL-16 is produced as a 67 kDa pro-IL-16 (Baier et al., 1997) that subsequently is cleaved by caspase-3

producing a 17 kDa secreted form of the chemokine that aggregates to form biogically active homotetramers (Zhang et al., 1998). IL-16 is chemoattractive for CD4+ T cells, eosinophils, and monocytes through a mechanism involving binding to CD4 (Zhang et al., 1998), although recent data suggest that CD4 is not the only receptor for IL-16 function (Mathy et al., 2000). In addition to its chemotactic function, IL-16 induces the expression of the IL-2 receptor alpha chain and MHC class II molecules (Cruikshank et al., 1987). Recently, a cDNA from an expressed sequence tags (EST) cDNA library, prepared from intestinal IEL of Eimeria-infected chickens and containing a full-length open reading frame (ORF) of pro-IL-16, was characterized (Min and Lillehoj, 2004). The encoded protein, predicted to consist of 607 amino acids, showed 86% sequence homology to duck pro-IL-16 and 49-52% homology to various mammalian homologues. By Northen blot analysis, IL-16 transcripts were identified in chicken lymphoid tissues, but not in the non-lymphoid tissues examined. A recombinant protein containing the COOH-terminal 149 amino acids of pro-IL-16 when expressed in COS-7 cells showed chemoattractant activity for splenic lymphocytes.

IL-17 was cloned originally from an activated T cell hybridoma produced by the fusion of a mouse cytotoxic T cell clone with a rat T cell hybridoma, and referred to as CTLA-8 (cytotoxic T lymphocyte-associated antigen 8)(Rouvier et al., 1993). IL-17 was produced in a mixture of glycosylated (22 kDa) and non-glycosylated (15 kDa) forms and secreted by activated CD4+ T cells as covalently bound homodimers (Fossiez et al., 1996). Whereas IL-17 transcripts were restricted to activated T cells, their receptors were found to be expressed ubiquitously in a variety of mammalian tissues and cell lines (Yao et al., 1995,1997). Functional studies indicated that IL-17 is involved in a broad range of cellular activities. For example, IL-17 stimulated osteoclastogenesis (Kotake et al., 1999), granulopoiesis (Schwarzenberger, et al., 1998), and T cell proliferation by suboptimal concentrations of phytohemagglutinin (Yao et al., 1995). Chicken IL-17 was cloned from an EST cDNA library prepared from intestinal IEL of Eimeria-infected chickens (Min and Lillehoj, 2002). It contained a 507 bp ORF predicted to encode a protein of 169 amino acids with a molecular mass of 18.9 kDa, a 27 residue NH2-terminal signal peptide, a single potential N-linked glycosylation site, and 6 cysteine residues conserved with mammalian IL-17s. Chicken IL-17 shared 37-46% amino acid sequence identity with the previously described mammalian homologues and also was homologous to ORF 13 of Herpes virus saimiri (HVS 13). By Northen blot analysis, IL-17 transcripts were identified in a reticuloendotheliosis virus-transformed chicken lymphoblast cell line (CU205) and Con A-stimulated splenic lymphocytes, but not other chicken cell lines or normal tissues. Conditioned medium from COS-7 cells transfected with the chicken IL-17 cDNA induced IL-6 production by chicken embryonic fibroblasts suggesting a functional role for the cytokine in avian immunity.

Tumor necrosis factor (TNF)-a or b have not been well characterized in poultry at present. However, macrophages obtained during and immediately following an infection with E. maxima or E. tenella produced a TNF-like activity in a biphasic fashion, whereby the first peak was associated with the pathogenesis of disease and the second peak with the development of a protective immunity (Byrnes et al., 1993). The production of significantly greater amounts of TNF during day 3-6 after inoculation correlated with the appearance of the most characteristic local and systemic pathophysiological changes induced by coccidia (Byrnes et al., 1993). Zhang et al. (1995a,b) investigated the effect of a TNF-like activity on the pathogenesis of coccidiosis in inbred chickens. The TNF-like factor was produced by peripheral blood macrophages in time- and dose-dependent manners following primary, but not secondary, E. tenella infection. Treatment of chickens with antibody against TNF resulted in a partial abrogation of E. tenella induced body weight loss in SC chickens.

Transforming growth factor (TGF)-b is a pleiotropic anti-inflammatory cytokine that stimulates the repair of damaged mucosal epithelial integrity following injury (Robinson et al., 2000). Lymphocytes that secrete TGF-b downregulate host immune and inflammatory responses, especially in the intestinal mucosa (Strober et al., 1997). The expression of TGF-b2, 3, and 4 was investigated using cDNA probes and antibodies specific for the different TGF-b isoforms in chickens (Jakowlew et al., 1997). After infection with E. acervulina, expression of TGF-b4 mRNA which is equivalent to TGF-b1 in mammals, increased 5- to 8-fold in intestinal IEL and 2.5-fold in spleen cells, whereas the expression of mRNA for TGF-b2 and TGF-b3 remained constant in these cells. Administration of TGF-b to T. gondii-infected severe combined immunodeficiency (SCID) mice resulted in an earlier mortality and shortening of the survival time of mice given exogenous IL-12. Administration of anti-TGF-b to SCID mice beginning 4 hr prior to infection and every 2 days thereafter prolonged the survival time significantly. These data demonstrated the ability of TGF-b to antagonize IL-12-induced IFN-g production by SCID mice and suggested a role for TGF-b in the regulation of T cell-independent resistance mechanism to T. gondii (Hunter et al., 1995).

IL-6 is a pleotropic lymphokine originally described as a T cell-derived lymphokine that induced the maturation of B cells into antibody-producing plasma cells (Narazaki and Kishimoto, 1994). Chicken IL-6 shows about 35% sequence identity to human IL-6 (Schneider et al., 2001). Bacterially expressed chicken IL-6 carrying a histidine tag in place of the signal peptide was biologically active and induced the proliferation of the IL-6-dependent murine hybridoma cell line 7TD1 (Schneider et al., 2001). Production of chicken IL-6-like factor activity was detected by a murine IL-6 7TD1 bioassay in serum taken from chickens infected with E. tenella during the course of a primary infection (Lynagh et al., 2000). IL-6

activity was detected during the first few hours post-infection indicating a possible role of this cytokine in the development of acquired immunity.

In vitro production of IL-1 by macrophages obtained from Eimeria-infected chickens was observed during and immediately following infection with E. maxima or E. tenella (Byrnes et al., 1993). Lymphocytes from Eimeria-infected chickens produced a higher level of IL-1 following stimulation than cells from non-infected birds. RT-PCR measurement of IL-1 production demonstrated a 27- to 80-fold increase in the IL-1b transcript levels at day 7 after infection with E. tenella and E. maxima (Laurent et al., 2001). The precise role of IL-1 in the development of resistance against coccidiosis needs to be better characterized in view of its documented role in various infections. Chemokines are important mediators of cell migration during inflammation and in normal leukocyte trafficking. These proteins are generally active at the nanomolar concentration and are produced by a wide variety of cell types in response to exogeneous irritants and endogeneous mediators such as IL-1, TNF, PDGF, and IFN-g (Oppenheim, 1991). Chemokines are grouped into four structural families characterized by the position of their amino-terminal cysteine residues (Zlotnik and Yoshie, 2000). The CXC class, which has one amino acid separated by two cysteine residues, and the CC class, which possess two consecutive cysteine residues, is the most common chemokines. IL-8 and K60 are CXC chemokines (Kaiser et al., 1999; Sick et al., 2000) and K203 is a CC chemokine recently cloned from chickens (Sick et al., 2000). The K203 cDNA cloned from the chicken macrophage cell line HD-11 stimulated with LPS, revealed 50% sequence identity to the mammalian macrophage inflammatory protein 1b (MIP-1b) (Sick et al., 2000). Laurent et al. (2001) showed that mRNA levels of the CC chemokines K203 and MIP-1b were upregulated 200- and 80-fold, respectively, in the cecum in response to E. tenella infection, and 100- and 5-fold in the jejunum in response to E. maxima infection. Interestingly, no discernible changes were observed in the mRNA levels of the CXC chemokines IL-8, and K60.

The role of various cytokines and chemokines needs to be better studied to understand how these different factors interact to eliminate parasites from the host and to develop memory responses against later infections. To accomplish this, we generated an EST cDNA library from IEL of Eimeria-infected chickens (Min et al., 2005) and have identified two new chicken cytokines, IL-16 (Min and Lillehoj, 2003) and IL-17 (Min and Lillehoj 2002). Both cytokines were elevated in Eimeria-infected tissues, they may be involved in regulating local immune response to coccidia. Analysis of 30 different cytokines and chemokines during coccidia infection indicated that cytokines invloved in mediating Th1 responses seem to be dominant during early times after coccidiosis (unpublished data) as best manifested by the proven involvement of IFN-g (Lillehoj and Choi, 1998; Lillehoj et al., 2000; Lillehoj et al., 2004; Lowenthal et al., 1997). The role of Th2 type cytokines should also be investigated in coccidiosis in order to obtain better insights on protective immunity. In toxoplasma infection, mice defective in IL-10, an anti-inflammatory cytokine, showed enhanced susceptibility to disease suggesting the role of IL-10 in downregulating inflammatory response to prevent host immunopathology (Gazzinelli et al., 1996). Recent evidence indicated that IL-10 is produced during coccidiosis (Rothwell et al., 2004), but its role in disease pathogenesis has not been investigated. Future studies to delineate cytokine regulation of local immunity to coccidia will lead to better understanding of host-parasite immunobiology and novel control strategies against coccidiosis.

FUTURE DIRECTIONS IN STUDYING HOST IMMUNE RESPONSE GENES CONTROLLING COCCIDIOSIS RESISTANCE USING DNA MICROARRAY

With increased information on poultry genomics and the availability of several tissue-specific cDNA EST libraries, high throughput gene expression analysis is possible to study host immune response to Eimeria (Min et al. 2005). DNA microarray is a revolutionary tool for genomic study of interested traits in a high throughput manner. 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 (Eisen and Brown, 1999). In one approach, the sample of interest, such as mRNA isolated from a certain tissue, is used to synthesize cDNA labeled with a fluorescent dyes. The labeled cDNA probe is then hybridized to the array and a post-hybridization image is developed. The color density of individual nucleic acid species reflects the relative amount of labeled cDNA hybridized to the DNA immobilized at the known position on the array. By comparing different samples tested in well-controlled conditions, changes in expression levels of individual genes can be detected. Once genes of interest are identified, Northern blotting or RT-PCR can be used to confirm genes with differential expression. The genes with significant differences can be used as potential candidate genes influencing disease susceptibility traits.

Development of chicken intestinal cDNA microarray to investigate host immunity against coccidia: We have established a normalized chicken intestinal cDNA library using pooled intestinal tissues from coccidia-infected chickens (Min et al., 2005). The library was prepared from intestinal epithelial cells and lymphocytes at 0, 1, 2, 3, and 4 days post-infection with Eimeria. According to the normalization control, the redundancy in this library has been reduced by 37-fold. Individual clones (n=34,078) were randomly picked and sequenced, generating 14,409 chicken-specific ESTs that could be grouped into 9,446 unique contigs. The majority of contigs (7,595; 80.4%) consisted of single ESTs and the remaining

1,851 contigs were composed of clusters of 2 or more overlapping/identical ESTs (average of 3.7 ESTs per cluster contig). Most cluster contigs (1,567; 84.7%) contained 2-4 ESTs comprising 30.6% (4,418) of the total number of ESTs. The contig average readable sequence length was 418 bp with almost half (45.8%) falling within the range of 400-600 bp and the average sequence length per contig was 712 bp and 71.2% (1,318) of all contigs ranged from 0.5-1.0 kb.

Using the Basic Local Alignment Search Tool (BLAST) program to perform sequence-similarity searches against the GenBank nucleic acid sequence database, we analyzed the 18 cluster contigs containing 15 or more ESTs, which could be regarded as abundant transcripts and therefore were most likely to match previously described genes. This group of contigs constituted 1.0% of the total number of clusters and 3.6% of the total ESTs. Ten sequences were highly homologous to previously described chicken genes. They included NK-lysin, apolipoprotein AIV, fatty acid binding protein, acid ribosomal phosphoprotein, a-tublin, GAPDH, and ferritin heavy chain. Comparison of our intestine cDNA sequence data with chicken DNA sequences in GenBank identified 125 clones which encoded novel genes. Of these, 110 genes were unknown and the remaining genes showed weak homologies to CC chemokine receptor type 8, cell adhesion receptor CD36, unc-51-like kinase 1, death-associated protein kinase 2, molybdenum cofactor sulfurase, lysosomal alpha-glucosidase precursor, FYVE and coiled-coil domain containing 1, NADH-ubiquinone oxidoreductase 23 kDa subunit, cytochrome b, proline rich protein 2, XE7 protein, glypican-5 precursor, nuclear receptor ROR gamma, and LPS-induced TNF-a factor. These EST sequences from Eimeria-stimulated intestinal IEL transcripts will be used to study global gene expression profiling and to identify novel immune-related genes during avian coccidiosis and in other enteric diseases of poultry.

Analysis of cell-mediated immune response to Eimeria using T lymphocyte cDNA microarray: In view of importance of T cell-mediated immunity in coccidiosis resistance, we initially selected 450 clones encoding immune response associated genes from a cDNA library prepared from mitogen-activated T lymphocytes (Min et al., 2003). To assess the changes in intestinal gene expression of chickens infected with E. acervulina or E. maxima, IEL were collected from the duodenum or ileum at 1, 2, 3, and 4 days following primary or secondary infection. In general, E. acervulina primary and secondary infection resulted in up- or down-regulation of more transcripts compared with E. maxima infection and primary infection by either parasite induced changes in a greater number of transcripts compared with secondary infection (Min et al., 2003). Specifically, E. acervulina and E. maxima infection affected the levels of 99 and 51 gene transcripts respectively following primary infection and 46 and 25 transcripts following secondary infection. Conversely, E. acervulina and E. maxima decreased the levels 88 and 56 gene transcripts respectively following primary infection and 22 and 37 transcripts following secondary infection. When considering all time points examined following primary or secondary infection with E. acervulina or E. maxima, the quantities of 5 gene transcripts were commonly induced (CMRF35 leukocyte immunoglobulin-like receptor, zinc finger gene, PmbA homolog, granulysin precursor, cyclophilin A) and 8 were repressed (alpha-actinin, hypothetical protein F39H12.5, spleen mitotic checkpoint BUB3, interferon-induced granylate-binding protein 2, transcription factor NF-YC subunit, transport associated protein 3, alpha-adaptin, homobox protein HOX-D8).

Since the changes in cytokine genes following Eimeria infection is an indication of local cell-mediated immunity (Lillehoj et al., 2003), we included in our microarray analysis 12 cytokine genes to monitor changes in their corresponding transcripts subsequent to Eimeria infection. The transcript levels for IL-8, IL-15, and lymphotactin genes were increased at all time points examined following primary or secondary infection with E. acervulina or E. maxima, whereas IL-18 and osteopontin gene transcripts were repressed (Min et al., 2003). With the exception of TGF-b4, changes in the levels of all cytokine transcripts examined were similar when comparing primary infection of E. acervulina with E. maxima. Similarly, after secondary infection, all transcript levels except those for IL-6 and TGF-b4 showed comparable changes when comparing E. acervulina with E. maxima.

CONCLUDING REMARKS

In view of increasing consumer's concern about drug residues in the food supply and impending regulations on the use of growth promoting drugs in poultry production, the industry will eventually look for alternative method for coccidiosis control. Problems associated with antigenic variation of field strains and the cost of producing multiple-species live vaccines pose limits on the current vaccination approaches. Thus, novel strategies to control coccidiosis are needed, but this will only be realized after a systematic and detailed analysis of host-parasite interactions at the molecular and cellular levels are completed. In particular, fundamental knowledge on the basic immunobiology from initial parasite invasion to intracellular development and ultimate elimination from the host is very limited. Increasing evidence shows the magnitude of complexity involved in host immune responses to Eimeria. Additional basic research is needed to ascertain the detailed immunological and physiological processes mediating protective immunity. The need to continue seeking more effective ways to minimize the impact of poultry coccidiosis is undisputable, but critical resources are severely lacking making it difficult to effect timely progress. Encouraging results obtained from recent molecular and immunological studies that

show the ability of dietary modulation on intestinal immunity and enhanced disease resistance against enteric pathogens of economic importance need to be further explored. One encouraging finding is the feasibility to induce protective immunity against live parasites using recombinant vaccines delivered in ovo. The performance of these novel vaccines will have to be verified in field evaluations in commercial settings.

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