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A Review: Host Immune Response to Coccidiosis in Poultry

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Coccidiosis

Coccidiosis is an enteric parasitic disease caused by a protozoan belonging to the phylum Apicomplexa and genus Eimeria. Coccidian infection generally damages the lining of the epithelial cells of the gastrointestinal tract, thus resulting in the interruption of feed intake, digestive processes, and nutrient absorption. In severe cases, there may be systemic effects such as high fecal blood loss, severe shock syndrome and even high mortality may occur. In commercial poultry there are several *Eimeria species* that are of high importance, and they are as follows: *E. acervulina*, *E. mivati*, *E. maxima*, *E. tenella*, *E. necatrix*, *E. brunetti* and *E. mitis*. Each of these species is certainly unique and can be specified by various means. All *Eimeria species* in poultry are very host and site specific [1]. For example, in chicken, *E. acervulina* generally affects the upper region of the intestine, particularly the duodenal loop. *E. mivati*, is also found in the upper region but can spread down the intestines. *E. mitis* is located and attacks the lower intestine but does not colonize as found in other species. *E. maxima* are primarily found in the mid intestine near the yolk sac diverticulum. *E. tenella* is only found in the cecal pouches of the gastrointestinal tract and generally creates a multitude of problems in the ceca. *E. brunetti* is found in the lower section of the intestine while *E. necatrix* lesions are found in the middle intestine but have sexual stages in the cecal pouches [1]. However, one exception to the host specificity is demonstrated by *E. meleagrimites*, which cause serious damage in the epithelial cells in the mid-gut region, especially turkeys [1]. It was also speculated that the underlying mechanism of the host is not well understood but most likely includes immune status, nutritional and biochemical factors. Genetic factors may play a role in determining which host is affected by the *Eimeria species*.

Coccidiosis generally affects younger birds more severely and is most prevalent and destructive in intensively reared commercial poultry production systems, perhaps because of; high stocking density, poor litter management and general sanitation. The general signs of the disease include reduced feed consumption, poor feed conversion efficiency, decreased growth rate, severe diarrhea (sometimes bloody feces as in the case of *E. tenella* infection), ruffled feathers, morbidity, thriftiness, dehydration, and mortality in severe cases [1].

Transmission of the disease generally occurs where the birds consume sporulated oocysts. Since birds are coprophagous in nature, they constantly have access to fecal matter especially when grown on a deep litter system. These conditions lead to re-infestation problems. Transmission can also occur when chickens encounter wild birds, rodents, pets, and contaminated feeding equipment. However, it is shown to be more highly transmissible when individuals (humans) move from house to house and from farm to farm or by other mechanical means [1].

Life Cycle of Eimeria

The life cycle of Eimeria is a rather complex one when compared to other intestinal parasites. The life cycle consists of three principal processes: a schizogony (asexual) phase, gametogony (sexual) phase and a sporogony (asexual) phase. These phases are further divided into exogenous (outside of host) and endogenous (within host) phases [2].

The infected host excretes unsporulated oocysts thus initiating the exogenous phase of development. In the presence of oxygen, sporulation of the oocysts generally occurs outside of the host. The unsporulated oocyst divides into two mature sporozoites that were from four sporocysts. The sporulated oocyst is then ingested by the bird where the oocyst wall is softened by the action of digestive enzymes from the bird's stomach and crushed by the action of the gizzard. This crushing action releases the sporozoites which then invade the mucosa of the intestine and begins the cell cycle within the intestinal epithelial cells. However, different species of Eimeria vary in the intestinal site of infection and the cell type infected. After penetrating the cell, the sporozoites develop to become trophozoites and further meront into a merogony. For the case of *E. maxima* and *E. tenella*, the transportation goes from the epithelium down to the lamina propria and lastly, the crypt epithelium for further development [2].

Through sexual schizogony and other frequent discharge of the merozoites that take over other calls and also initiate other merogonic cycles, the merozoites formation occurs. The amount of merogonic cycles which occur are determined genetically and are usually not the same for each species. Multiplication of merozoites within the host is genetically limited and its production is constant. Gametogony is a process at which merozoites from the final generation invade intestinal cells and develop into macro- and micro gamonts. The macrogamonts mature into large macrogametes while the microgametes are spited to form various flagellated microgametes. The macrogametes are penetrated and fertilized by the microgametes and then form a zygote and an unsporulated oocyst [1]. A study by Rose [2] showed that the unsporulated oocyst breaks out of the host cell by further passing into the feces. However, when there is no re-infection, within 7-14 days depending on the kind of species, the parasites generally cleave from its host [2].

Effects and Severity of Coccidiosis in Chickens

An alteration of the intestinal mucosa that may give rise to other enteric diseases is caused by Eimeria which is an infection of the gastrointestinal tract. One primary example is the proliferation of *Clostridium perfringens*, which causes necrotic enteritis, *Salmonella*, *E. coli* and *Campylobacter*. Primarily, coccidiosis is a self-limiting disease of young birds in which re-exposure is important for the maintenance of immunity [2]. The severity of coccidiosis in the avian species is generally affected by external factors. These factors include management, stress, environment, and climate. However, research by Ruff [3] showed that only a few chronic diseases occur with public poultry houses which have relatively high humidity and temperatures.

Despite the external factors mentioned, internal factors including age, genetics, disease interactions, nutritional status and immunity may affect the severity of the disease. Disease susceptibility, especially in the case of Eimeria infection, depends on the host genomic make up. Also, breeds and individuals within breeds are different in terms of susceptibility to coccidiosis. These similarities occur because of variations in random genetics, strains, and breeds. It was also demonstrated in a similar experiment that resistance to the Eimeria infection correlates with the strong proliferative response of the lymphocytes, primarily $CD8^+$ and $\gamma\delta$ TCR^+, to parasite antigens in resistant, but not in susceptible breeds. The authors also showed that $CD8^+$ cells were primed by primary infection, divided rapidly upon challenge, and migrated to mediate in resistance in the intestine [3]. However, mortality from coccidiosis decreased with age and Eimeria infection was generally more severe in young birds partly due to low resistance because of immature immunity. Other infections and diseases like Marek's and Infectious Bursal Disease (IBD) may also cause a rise in the severity of coccidiosis. Nevertheless, developing specific immunity after primary infection may cause a substantial reduction in the severity of coccidiosis after subsequent infections [3].

In the poultry industry, the cost of preventing and treating coccidiosis has increased tremendously due to development of prophylactic and therapeutic drugs. However, these drugs also require the approval by the Food and Drug Administration (FDA), which requires a substantial amount of time for testing and verification. CAST (2002) estimates that the cost of preventing and treating coccidiosis in poultry was approximately 1.3 billion $US annually in the United States and Canada. The prevalence of the disease is still widespread in the poultry industry because of the continuous increase in the issue of drug resistance of the parasites, irrespective of the integrated management systems in use.

Control of Coccidiosis

In the poultry industry, application of anticoccidials (mostly in-feed application), vaccination (generally ocular application at the hatchery) and management of the poultry house, are all employed as control methods against Eimeria infection. Methods such as disinfection and fumigation are generally not effective strategies in controlling Eimeria infection. However, practicing good sanitation helps in the mechanical removal of oocysts in the environment and further decreases exposure of the birds. In addition, complete reliance on this method solely is not advisable as it is not effective in reducing disease [4]. Prophylactic chemotherapy has proven very successful and has been fundamental for the growth of the poultry industry. Since anticoccidial drugs began in the late 1940's many coccidiostats and coccidiocidal compounds have been introduced, many of which have antibiotic and growth promotion properties. Chemotherapeutic drugs used in recent times are synthetic classified while through microbial fermentation, antibiotic compounds and ionophores are produced. However,

chemotherapeutic strategies have to do with the total suppression of disease (in broiler flocks especially), including the treatment of coccidian outbreaks and partially suppressing diseases to allow for the development of immunity (layer flocks). Currently, there's no single drug that's completely effective for the entire three roles [4].

The futuristic approach of the use of chemotherapy for the suppression of Eimerian infection is still questionable mainly due to the widespread emergence of drug resistance in the parasites and to the expensive costs for further characterization, discovery, the development, and the registration of new drugs. Given the heavy use of anticoccidial drugs for many years, it is not surprising that Eimerian resistance to anticoccidial drugs is increasing in all markets [4]. In general, anticoccidial resistance occurs because of continuous reliance on the use of suboptimal levels of drugs, medication, and small life cycle of the parasites. The characteristics of the parasites, the fast asexual multiplication cycle, and the sexual recombination of their life cycle contribute substantially to the development of drug resistance. Analysis by Random Amplification of Polymorphic DNA (RAPD) was used by Girard et al. [4] to examine possible genetic correlation of drug-resistant and drug-susceptible parasite strains showed that parasite strains with different patterns of susceptibility were found to be genetically different, possibly resulting from point mutations.

The development of drug resistance in Eimeria parasites has prompted the improvement of other means of control Natural alternative means of controlling Eimeria infection has been sought to date primarily because of the banning of antibiotic and growth promoter drugs used in poultry production by the European Union (EU). This was based on the premise that these drugs can have serious residual effects in meats and subsequently end up in the human food chain. Natural alternatives include yeast cell wall, yucca, garlic, and neem. The main current alternative is immunological control through vaccination since it shows the most promising sign of minimizing the effects of Eimeria infection [5].

One major primary means of these vaccines is to produce specific immunity for each species by using controlled inoculation with the oocysts of the species. This makes it easier for producers to control the size of the initial exposure of the birds to the parasites, as well as the strains of parasites used. In this regard, four different vaccines are currently available for the control of coccidiosis. Coccivac™ (Schering-Plough Animal Health) and Immucox™ (Vetch Laboratories) contain virulent forms of seven *Eimeria species*. Paracox™ (Schering-Plough Animal Health) contains 8 precocious lines (shortened life cycles) of the parasites, including seven species with two strains of *E. maxima*. Livacox™ (Biopharm) contains attenuated lines of *E. acervulina*, *E. maxima*, and *E. tenella*. Selection of precociousness is developed through attenuated lines of the parasites [5]. This trait is heritable, stable and has a lower pathogenicity and higher sensitivity to anticoccidials. The degree of protection afforded through vaccination depends on the amount and frequency of initial exposures, the species used and the immune status of the hosts. It was also shown that the method of vaccine delivery influences vaccine efficacy. Immucox™ was administered to 1-day-old chicks by oral gavage, gel delivery (oral), spray-cabinet (aerosol) and slurry (oral) delivery. Protectin, which is often measured by reduced lesion scores, weight increase, and reduced oocyst output was discovered to be high following administration of the vaccine by gel delivery orally [5].

Vaccines are commonly used in the breeder/replacement layer industries, but not in the broiler/heavy rooster industry. Vaccine is not used in broilers because of the decrease in the weight following vaccine administration. Exposure to the parasite must occur by 1 day of age for effective development of protection in broilers. Vaccines comprising attenuated species would be best suited to broiler flocks once an early delivery method has been developed. The administration of Paracox™ vaccine to broilers was compared to a drug shuttle program using halofuginone/salinomycin or nicarbazin/monensin [5]. Feed and water consumption were found to be the same between vaccinated and medicated groups but more deaths and disease outbreaks due to Eimeria were observed in the medicated houses.

The use of vaccination in conjunction with drug administration may extend the usefulness of anticoccidials. Sensitive strains, such as attenuated lines contained in the vaccines, compete with established drug-resistant strains. In the absence of medication, introduction of drug-sensitive parasites into houses where resistant parasites are dominant favours the replication of drug-sensitive parasites. The replacement of drug-resistant parasites with drug-sensitive parasites may permit more effective use of drugs after the period of vaccine use. Concurrent application of vaccines and chemotherapy may also be beneficial in extending anticoccidial usefulness or in alleviating possible pathogenic effects associated with administration of non-attenuated vaccines (Chapman, 1999).

Maternal immunization

The replication, shedding of parasites and the reduction of flock exposure, can be reduced by maternal vaccination. Antibodies, including IgG (yolk), IgA and IgM (yolk and egg white) are secreted as egg formation occurs. Protective antibodies were transferred to chicks from hens immunized with gametocyte antigens (56, 83 and 250kDa). Chicks born to immunized hens have reduced oocyst output which increases with age as the maternal antibodies are decreased. Antibodies to these surface antigens may limit development of disease through inhibition of the development, growth, as well as the fertilization of gametes [6].

Maternal immunization protects the chicks from disease from the time of hatching and is economical as immunization protects all offspring from one hen; up to 100 chicks as increase in oocyst number in the litter of poultry houses occurs between 3- and 5-weeks post-hatch. Thus, protective effects lasting only 3 weeks would be beneficial in decreasing the degree of seeding and accumulation of oocysts in the litter. The primary effect of maternal antibodies was disruption of oocyst development and decreased oocyst outputs following infection. The reduced numbers of oocysts in the environment nevertheless elicit active immune responses while reducing the severity of disease. Research into recombinant

vaccines based on protective antigens of *Eimeria spp*. is ongoing but these vaccines are unlikely to be available soon [6].

Diet

Several feedstuffs and natural products have been tested for their possible protective role against coccidian infections when used as food additives.

Omega's three fatty acids, particularly Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) are present in high amounts in flaxseed. Increased use of flaxseed in the diet of young chicks decreased parasitization of ceca by *E. tenella*. This flaxseed diet, however, did not have any effect on infections with *E. maxima* or *E. acervulina* [5]. It is possible that *E. tenella* was susceptible to the oxidative stress the fatty acids produced in the anaerobic niche that the parasite occupies in the ceca, since the supplement did not have an effect in *Eimeria spp* that occupy well-oxygenated parts of the intestine.

Betaine has also been considered as a food additive, because of its osmo-protectant properties. Coccidiosis causes osmotic stress in the intestine of birds because of diarrhea and resulting dehydration. Betaine has been shown to considerably ameliorate infection by restoring crypt-villus ratio and stabilizing in this way the structure of the intestinal mucosa. Additionally, betaine decreases lesions in the infected intestine [6]. Significantly better weight gains have been noted in birds fed a diet supplemented with betaine and then challenged with virulent parasites when compared to similarly challenged birds that were fed control feed [6]. The outcome of coccidiosis did not significantly alter by supplementing feeds with vitamins A, B and C.

Host Immune Response to Coccidiosis

The host main immune responses to *Eimeria spp*. are to reduce parasite multiplication and to decrease the adverse effects of parasites. Many factors influencing parasite immune response are species of Eimeria, stage responsible for intestinal damage, the number of oocysts ingested, host age, genotype, and general health. The degree of protection in the host following primary infection depends on the time interval between initial and infections as well as species and stage specificity of the immune response that was elicited.

Host defense mechanisms are activated following sporozoite invasion in the gut. Many structural and functional changes observed in local tissues following infections are the result of host response. Smooth muscle layers thicken due to cell proliferation. Epithelial responses include villus atrophy, crypt cell hyperplasia, increased fluid secretion, decreased absorption of nutrients and fluids and decreased gut enzyme activity. Three to four hours post-infection a local inflammatory reaction occurs; this reaction increases in severity following subsequent infections. Sporozoite invasion causes increased intestinal permeability, which leads to plasma leakage from vasculature, coinciding with cell invasion.

Macrophages, B-lymphocytes, T-lymphocytes, soluble factors, following sporozoites invasion, leukocytes, and other components of Gut-Associated Lymphoid Tissue (GALT) could be found at the infection site [7]. Phagocytosis helps activated macrophages modulate severity of infection, reducing sporozoites and merozoites numbers. Antibody and Cell Mediated Immunity (CMI) is introduced by $CD4^+$ T-cells. Limited invasion, inhibition of sporozoite, merozoite development, inducing parasite death followed by removal of host cells, and culminating in parasite elimination affects the innate immunity of the parasite at an early stage [2].

Influence of MHC

The immune response genes found in the B locus (MHC) of chicken influence responsiveness to antigens and development of disease, including, for example Marek's disease. The importance of these genes, however, in susceptibility to coccidiosis is not well known but strain differences in resistance are known to occur. Two chicken strains differing in B haplotype [SC (B^2B^2) and FP ($B^{15}B^{21}$)] were infected with various *Eimeria spp*. The FP strain was found to be more susceptible to *E. tenella*, *E. acervulina* and *E. maxima* than strain SC. The antibody responses were similar following infection with each strain but differences in CMI and antigen recognition were described by Lillehoj [8]. Other genes are believed to play more dominant roles in the development of resistance to Eimerian parasites. These genes are not in the B locus nor are they linked to the locus.

Antibody Responses to *Eimeria spp.*

Antibody responses play a minor role in protecting immunity to *Eimeria spp*. and have only minimal control infections. Infection with *Eimeria spp*. induces an antibody response, likely against invasive stages of the parasite. It is likely that antibodies may play a role only in early infection [7]. Immunity to *Eimeria spp*. was assessed in B-cell and T-cell-deficient chickens. Oocyst production was increased in bursectomized chickens as compared to control chickens. However, the birds were resistant to re-infection, although resistance was lower than in controls. The authors concluded that T-cells were important effectors of resistance, and those B-cells were involved only to a minor degree. Passive transfer of immune serum resulted in variable and minor resistance to re-infection, again supporting a minor role for antibodies.

Parasite-specific serum IgM and IgG and biliary IgA increased 7 days post-primary infections with *E. acervulina*, *E. tenella* and *E. maxima*. There were no significant increases in antibody titers following secondary infections [8]. In a separate study, only an increase in IgA followed by primary infection with *E. tenella* was observed. This immunoglobulin increased on days 6 and 10 following infection and the authors speculated that the increase on day 10 represented a parasite-specific response. Following secondary infection, IgA increases to a lesser extent and occurs 10 days post-infection [8]. However, increases in IgM^+, IgG^+ and IgA^+ plasma cells were observed following infections with *E. tenella*. In naïve chickens, IgM^+ plasma cells increased more than IgG^+ cells,

whereas the opposite was true following challenges of immune birds. In naïve and immune birds, intestinal IgA$^+$ plasma cells increased more than other isotopes following infection [9].

Immunoglobulins A and M are abundant in intestinal secretions of chickens and IgG is believed to occur in intestinal sites after being derived from serum or following leakage from the lymphatics [7]. The production of local antibodies following *E. tenella* infection was determined through calculation of antibody release in intestinal culture supernatants. Serum IgM and IgG antibody decreased 10 days post-infection; an increase in IgA antibody in serum was not detected. Significant production of all three classes of antibodies by cells from infected tissues was observed. They also stated that the increase in IgA observed represented local production of the antibody whereas the other two classes were serum derived.

Antibody production following infection with an upper intestinal species (*E. acervulina*) and with a cecal species (*E. tenella*) was observed in the serum and locally at the site of infection. A rapid increase in IgA, IgM and IgG antibodies in serum was observed following infection with both species. Significant increases in IgA were observed in mucosal tissue from the duodenum and cecum 14 days post-infection following infection with *E. acervulin*. Immunoglobulin M increased by 7 days post-infection and then decreased [8]. A more delayed IgG antibody response was observed late in the second week following infection. Elevated IgA antibody was observed in the ceca 14 days following infection with *E. tenella*. IgM and IgG antibody responses were like those observed with *E. acervulina*, with higher antibody titers found at the cecal sites of infection. The amounts of IgG antibody were observed to remain elevated for the greatest time compared to the other 2 classes suggesting that the antibody was more important to local protection [8].

Increases in IgA, IgM and IgG antibodies in intestinal washes following infection with *E. maxima* have also been observed. The intestinal IgA antibody response occurred before IgM and IgG, suggesting that local IgA antibodies may be involved during infections with Eimeria. However, increases in serum IgG and IgM antibodies generally occurred prior to that of serum IgA antibodies [8].

Increased secretory IgA (sIgA) is often observed following infection with *Eimeria spp*. The duty of IgA during infection is not completely clear, however, it is believed to be insignificant. Chickens that lack isotopes are resistant to infections [7]. Its major duty could be in the reduction or prevention of invasion and possibly the inhibition of sporozoite development. Several mechanisms for the prevention of cell penetration by the sporozoite have been postulated. Blockage of sporozoite binding to host cells by antibody attachment to parasite surfaces may lead to direct blockage, induction of conformational change or reduced motility and steric hindrance.

Cell-mediated responses to Eimeria

For the development of protective immunity to occur, a response that involves macrophages, T-lymphocytes, mast cells and natural killer (NK) cells must be elicited. Both CD4$^+$ and CD8$^+$ T-lymphocytes are involved. After primary infection, CD4$^+$ cells are involved primarily in assisting antibody synthesis and in delayed-type hypersensitivity (DTH). CD8$^+$ cells are considered primarily responsible for anti-parasite responses during secondary infections, e.g., cytotoxicity [8]. No direct role for CD8$^+$ IELs has been observed. Numbers increase following challenge, and they are often observed in contact with infected cells suggesting that they are important in local defense and may be involved in cytotoxic attack [10].

Transfer and depletion experiments have shown the importance of T-lymphocytes in meditating resistance. Adoptively transferring *Eimeria spp*. with T-cells from immune birds is usually possible. Studies have shown that when cyclosporine was administered prior to primary parasite inoculation, susceptibility was increased. Administration of cyclosporine before secondary inoculation eliminated protective immunity [8]. The depletion of CD4$^+$ cells had no effect on the development of immunity. However, resistance to secondary infection, following the depletion of CD8+ cells was abrogated. It is concluded by the author that the role of CD4$^+$ cells during primary immune response was the production of interferon (IFN) and that the cell of CD8$^+$ was effective against infected epithelial cells. The infection of *E. tenella* and other diseases is reduced by macrophages. Macrophages mediate immune responses to the parasites through their role of antigen presenting cells. Furthermore, macrophages modulate responses by other cells of the immune system and the epithelial cells through the secretion of cytokines. Through the production of Reactive Oxygen Intermediates (ROI), Tumor Necrosis Factor (TNF), and Reactive Nitrogen Intermediates (RNI), they also function as effector cells, and all of these have antiparasitic effects. Kopko et al. [11] showed that the production of Nitrous Oxide (NO) by macrophages, which also has anti-parasitic effects, increased during infections with *Eimeria spp*.

Blood leukocytes increased following infection with *Eimeria spp*. Following primary infection with *E. maxima*, biphasic leukocytosis involving Large Mononuclear (LMN), Polymorphonuclear (PMN) cells, and lymphocytes occurred. LMN was possibly associated with lesion resolution and the highest increase was observed at the end parasite life cycle. Before and after peak oocyst production, an increase in PMNs was observed [11]. The appearance of sexual stages in the life cycle of the parasite decreases based on the numbers of leukocytes present. The leukocyte response in chickens re-infected with the parasite was rapid and involved increases in PMN and lymphocyte numbers. Observation of the infiltration of PMN and lymphocytes into the intestinal mucosa was done concurrently. Considering the problems of immune birds, circulating blood lymph's also known as primarily T-cells initially reduced and later increased [11]. To indicate the protective role of a population, the predominant involvement of the T-cells was taken. Circulating T-cells were involved in protective responses to *E. tenella*. Eight days following primary infection CD8$^+$ cells increased. This coincided with clonal expansion of lymphocytes in response to sporozoite antigens. A decrease in CD4$^+$ T-cells was observed at 9 to 10 days post-infection. Even though no changes in the lymphocyte

subsets were observed following challenge infection, Kopko et al. [11] concluded that CD8+ cells were important in the control of infection and to the induction of protective immunity.

Intestinal lymphocyte population studies, following infection with *Eimeria spp*, may be more informative. Lymphocyte responses occurring after infections with *E. acervulina*, *E. tenella*, and *E. maxima* have been described Lillehoj, [10] observed increased numbers of duodenal $CD4^+$ IELs from 4 days through 14 days post-infection with *E. acervulina*. Smaller increases were observed in both $CD8^+$ and γδ TCR^+ cells that increased by 12 days post-infection and decreased by 14 days. Following secondary infection with *E. acervulina*, $CD8^+$ T-cells increased. The author speculated that $CD8^+$ T-cells and NK cells were involved in protective immunity due to significant increases in these cells at the site of infection in resistant and not susceptible breeds of chickens.

Several authors have described intestinal cellular responses that occur following infection with the cecal parasite *E. tenella*. During a 24 to 96 hours post-infection of a naive chicken, an influx of macrophages and T-cells, which is predominantly, $CD4^+$ and $CD8^+$ was observed. The $CD4^+$ cells were thought to be involved with the induction of a cytotoxic response, MHC Class II recognition and the production of cytokines. Lymphocyte migration to the intestine was more rapid and involved mostly $CD8^+$ cells in immune chickens. More cells were observed in the lamina propria of these birds than in naive birds. The $CD8^+$ cells surrounded sporozoites in the lamina propria.

In a later study, Vervelde [9] observed similar changes in the intestinal lymphocyte populations. More T-cells were observed in the lamina propria following infection with *E. tenella* of immune birds than naive birds. Additionally, more $CD8^+$ lymphocytes were observed than $CD4^+$ lymphocytes in immune chickens. The reverse was observed in naive birds (more $CD4^+$ than $CD8^+$). Increases in $CD8^+$ cells but not γδ TCR^+ cells were observed following primary infection with *E. tenella*. There was an increase in $CD4^+$ cells 8 days post-infection and by day 14 it decreased. There is a difference in the early immune response of chicken to Eimeria parasites based on the site of infection when compared to the response of *E. tenella* with that to *E. acervulina*. Based on their findings, the number of $CD8^+$ lymphocytes appear relatively more elevated in distal regions of the intestinal tract.

Primary infection with *E.maxima* after one hour decreased local lymphocytes but later decreased after 3 to 7 hours. Also, T-cells rather than B-cells are most of the responding lymphocytes. Subsequent T-cell responses were biphasic, with increases in the number of $CD4^+$ cells in the lamina propria observed on day 3 and day 11 following primary infection [10]. Less response was observed in epithelial $CD4^+$ cells. In 0.5- and 3-hours challenge infection increase was observed in the lamina propria $CD4^+$. The CD8+ populations responded biophysically in lamina propria and intraepithelial regions, like $CD4^+$ cells. The numbers also increased following challenges, most notably in the epithelium. Distributions of αβTCR^+ and γδTCR^+ lymphocytes differed between the lamina propria and intraepithelial regions. A greater number of γδTCR^+ cells were observed in the epithelium than in the lamina propria. This population peaked on day 11 post-infection. Alpha beta TCR^+ cells were observed in both regions but in greater numbers in the lamina propria. These authors also speculated that $CD4^+$ and $CD8^+$ lymphocytes are involved in the development of specific immunity, but their roles were undescribed [10].

The decrease in NK cell activity was observed because of some primary infection with *E. maxima*. It was speculated that this was parasite-induced suppression, a strategy to enable parasite replication. Upon challenge, NK activity against chicken tumor cells increased in spleen and IEL cells [10]. These cells may influence the outcome of infection, perhaps by affecting parasite proliferation and elimination. The NK cells of the IEL region may also be involved in preventing invasion and may be involved in defense in this manner.

The importance of T-cells in mediating protection to *Eimeria spp*. has been established but the exact mechanisms underlying protection by these cells are not known. Increased production of cytokines may be important as increased levels have been observed during protective immune responses to *Eimeria spp*. The immune response in mucosal sites and the function of intestinal epithelial cells are regulated by cytokines produced during the inflammatory responses. The outcome of an infection may depend on the differential activation of T_H subsets, T_H1 and T_H2 presumed to occur in chickens by analogy with mammalian systems. Lillehoj et al. [7] found that soluble factors secreted by T-cells, presumed to be cytokines, inhibited intracellular development of parasites *in vitro* and were protective *in vivo*.

Resistance to Eimeria is believed to involve IFN-γ. Its effects may include alteration of host cells, rendering them unsuitable for parasite development through the induction of compounds such as ROI and RNI which are toxic to the parasites, Other anti-parasitic mechanisms may include the activation of Antibody-Dependent-Cell-Mediated Cytotoxicity (ADCC) and the promotion of the release of contents of perforin and protease containing cytoplasmic granules [12]. Avian IFN-γ has been cloned and expressed in prokaryotic and eukaryotic systems. It was found to have antiviral activity, activate macrophages, up-regulate MHC Class I and II expression, and increase macrophage production of NO. Interferon-gamma may also inhibit parasite growth and development through other mechanisms [12]. The development of *E. tenella* is inhibited by the recombinants IFN-γ in a reversible manner without causing sporozoite death. Furthermore, pretreatment of sporozoites with the cytokine inhibited sporozoite invasion of host cells. The cytokine acted on the host cell and not the parasite, possibly through the induction of membrane changes. Thus IFN-γ may play a protective role during infection with Eimeria through limiting host cell invasion and parasite development [12].

Production of IFN-γ has been observed following infection with different species of Eimeria. Cultures generated from spleen cells isolated from *E. tenella*-immune chickens released IFN-γ following antigenic stimulation 24 hours post-infection. Antigen-

induced release occurred at 35 days post-infection, when the development of immunity was complete. Reduced amounts of IFN-γ produced by splenic lymphocytes following infection with *E. maxima* were detected by 5 days post-infection, possibly due to immunosuppression. Normal amounts were observed 10 to 15 days post-infection, followed by a significant increase at 20 days post-infection, an earlier peak than that observed above [12].

In a separate study, increased concentrations of IFN-γ were obtained from intestinal washings at day 4 post *E. maxima* infection. Serum increases were not noted until day 8 to day 10 post-infection [12]. The early, local IFN-γ response suggested the importance of the cytokine in protective responses following primary infections. Differences in disease resistance between SC and TK chicken strains were correlated with differences in the early, local production of IFN-γ. Resistant SC chickens had elevated local IFN-γ when compared with the more susceptible TK birds.

There are two forms of IL-2 in chickens: a homodimeric and an aggregated form. The cytokine is relatively unstable, having a half-life of 10 hours. The cytokine may also be involved in the development of immunity to coccidiosis through effects on cytotoxic lymphocytes and NK cells. Chicken IL-2 is a growth factor for γδ TCR^+ cells and $CD8^+$ cells, stimulating their growth in culture. Local IL-2 produced during infections contributes to the local proliferation of T-cells. An increase in IL-2 mRNA occurs in the spleen and duodenum during infections with *E. acervulina*. Increases were also observed in γδ TCR^+ cells [13].

Tumour necrosis factor alpha (TNF-α) also has antiparasitic effects and increases in the blood following infection with Eimeria. Activated macrophages and NK cells are sources of cytokine [13]. A biphasic response was observed by Ovington et al. [14]. The early response was speculated to arise due to intestinal damage following infection and the second with the development of immunity to the parasites [15].

Conclusion

Immunity to Eimeria infections and response to Eimeria challenge in poultry are multifaceted. Different effector mechanisms may be involved based on the stages of the nutritional status of the birds, the genetic makeup of the host, prior to host exposure to parasite, and the parasite development within the cell. More research is needed to establish a detailed process by which protection against coccidiosis occurs. However, in the advent of new molecular techniques to manipulate the genome of the host pathogen, and a better understanding of the interactions between the gut associated lymphoid tissues and peripheral lymphoid organs, there will be a need to invent vaccines against enteric pathogens in the future. Avenues for the development of new control strategies against coccidiosis is because of the advanced interaction of nutrition, infection, and immunity. In addition, the development of molecular vaccines and recent evidence of cytokines to enhance host immunity to parasites makes biological factors feasible.

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