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Prospects for Immunological Intervention for Coccidiosis

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닭 콕시듐병의 면역학적 접근에 대한 전망

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요 약

콕시듐병은 감수성이 있는 숙주의 장에 주로 감염된 *Eimeria*에 의하여 일어나는 것으로 가축과 가금의 성장과 사료이용에 막대한 해를 미친다.

*Eimeria*속에 속하는 원충은 주로 세포내에 기생하며 무성생식과 유성생식을 거쳐야 하는 매우 복잡한 생활사를 가진다. *Eimeria*에 대한 백신개발은 주로 방어면역의 기전과 백신에 사용될 수 있는 항원을 찾는 방면으로 활발한 연구가 이루어지고 있다. 원충은 질병 본래의 모습이나 전파방법이 독특하므로 원충성 질병의 방제에 있어서는 이러한 점이 충분히 숙고되어야 할 것이다. 난해하고 복잡한 세포나 세포 분비물질간의 작용은 콕시듐병의 병성기전 뿐 아니라 방어 면역의 형성에도 매우 깊이 관여하고 있다. 따라서, *Eimeria*의 감염에 따른 방어기전을 충분히 이해하는 것이 가장 좋은 백신을 개발하는 첩경이 될 것이다.

I. INTRODUCTION

Due to the intense rearing of poultry, coccidiosis is still an economically important disease for industries worldwide. Coccidiosis is currently controlled by medication, but the increasing emergence of drug-resistant strains of coccidia urges development of alternative control strategies. The development of an effective coccidia vaccine should be feasible since natural infection by this parasite is self-limiting, displays minimum antigenic variation, and induces long lasting immunity. Recently, novel measures directed towards this goal have been utilized, including parasite attenuation and genetic engineering of coccidial antigens. Attenuated coccidia vaccines have been successfully produced through rigorous selection of precociousness. Precocious strains of all 7 species of *Eimeria* have been developed and vaccination programs using them are being actively pursued. Numerous recombinant coccidial antigens have been characterized and experimental immunization studies are promising. However,

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practical application of a recombinant vaccine to the poultry industry is still remote and a better understanding of mucosal immunity will be crucial before their application becomes meaningful. This review attempts to summarize recent studies on host immunity and vaccine development to coccidia. For more detailed information, the reader is referred to previous reviews on immunity(Rose, 1987; Lillehoj, 1991) and recombinant coccidial antigens(Ellis and Tomley, 1991).

1) IMMUNE RESPONSES TO COCCIDIA

A) Systemic responses to coccidia

Availability of various mAbs that detect cell surface antigens of chicken lymphocytes made it possible to analyze host immune responses to coccidia. Mouse mAbs that distinguish between chicken T_h and $T_{c/s}$ lymphocytes have been described(Chan et al., 1988; Lillehoj et al., 1988a). The CD4 and CD8 antigens are found on two main populations of T lymphocytes, T helper (T_h) and T cytotoxic/suppressor($T_{c/s}$) cells respectively. T_h cells appear to be the prime inducer population during many immune responses. T_h cells can be involved in the differentiation of B cells to antibody producing plasma cells, the induction of cytotoxic and suppressor activity mediated by other T cells, activation of macrophages, enhancement of NK cell activity, and the development of mast cells. $T_{c/s}$, when activated by T_h cells, perform suppressor or cytotoxic functions. CD4+ cells may occasionally mediate cytotoxic reactions and low levels of CD8 have been found on cells exhibiting NK activity. Recently two subpopulations of T_h cells have been defined based on their cytokine secretion profiles(Mosmann et al., 1986). T_h1 cells secrete *r*-IFN and also tend to secrete IL-2, while T_h2 cells secrete IL-4 and also tend to secrete IL-5, IL-6, and IL-10. The cell type whose secretions dominate may help to determine the outcome of certain parasitic infections. (Sher and Coffman, 1992). Coccidia-infected hosts show an enhanced cellular response to infecting parasites. An *in vitro* antigen-specific proliferation of T lymphocytes to oocyst antigens of *Eimeria* was observed. Various stages of *E. tenella* or soluble antigen were also able to induce proliferation of T cells taken from previously infected chickens(Lillehoj, 1986). *E. acervulina* antigens, however, induced little or no proliferation of *E. tenella*-immune T cells(Lillehoj, 1986) indicating that T cell immunity is specific for a species of *Eimeria*. However, cells from *E. acervulina*-immune birds responded equally well to both *E. acervulina* and *E. tenella*(Prowse, 1991) suggesting that some antigens of *E. acervulina* are cross-species reactive. The question still remains as to why birds immune to *E. acervulina* are susceptible to *E. tenella* infection.

In avian coccidiosis, the role of T cell in protection was studied in chickens treated with cyclosporin A(CsA), a drug that suppress cell-mediated immunity(Lillehoj, 1987a). *E. tenella*-immune chickens treated with CsA at the time of secondary infection, but not bursectomized chickens, were highly susceptible to challenge infection. However, when CsA was given concurrently with oocysts at the time of primary infection, resistance to infection was enhanced presumably due to cytotoxic effect of CsA on developing parasites(Lillehoj, 1987a). These studies indicate importance of cell-mediated immunity in the development of resistance to coccidiosis. A recent study showing an enhanced level of CD8-bearing T cells in the *Eimeria*-immune chicken intestine(Lillehoj and Bacon, 1991) suggests a role of cytotoxic cells in protection in chickens. CsA or DEX treatment of chickens prior to inoculation with turkey

Eimeria allowed those species to complete their life cycles(McLoughlin 1969), indicating that T cells are also involved in natural resistance to coccidia in a foreign-host.

Cytokines secreted in response to a general stimulus such as Con A or by specific antigen are important mediator of cellular immunity. Culture supernatants from Con A-stimulated lymphocytes reduced oocyst output of *E. acervulina* and *E. tenella* challenged chickens (Lillehoj et al., 1989a). These same supernatants were also capable of inhibiting the replication of parasites in MDBK cell cultures *in vitro*(Lillehoj et al., 1989a). It was also shown that macrophages were able to transfer sporozoites to the MDBK cells in culture and that treatment with sporozoite-stimulated culture supernatants eliminated this transfer ability(Lillehoj et al., 1989a). The culture supernatants had no direct cytotoxic effect on sporozoites(Lillehoj et al., 1989a).

Gamma-interferon(*r*-IFN), one of the cytokines present in supernatants of stimulated lymphocytes, has been implicated in immunity to coccidiosis. *r*-IFN is produced during coccidiosis(Prowse and Pallister, 1989).

Betamethasone and dexamethasone(DEX), drugs which suppress T cell activity, have been reported to enhance susceptibility to *Eimeria* infection(Long and Rose, 1970). Recent studies from this laboratory showed that DEX-treated chickens show enhanced CD4(T_h) to CD8($T_{c/s}$) ratio in spleen compared to untreated chickens (Isobe and Lillehoj, 1992a). Furthermore DEX-treated chickens show depressed cell-mediated immunity and low production of interleukin-2 and *r*-IFN. When DEX-treated chickens were infected with *E. mivati*, enhanced susceptibility with a prolonged patency was observed although coccidia specific IgG responses were enhanced(Isobe and Lillehoj, 1992b). These results suggest that interleukin-2 and *r*-IFN are important in primary infection.

Isotypes of anti-coccidia antibodies in both the circulation and mucosal secretions have been studied(Trees et al., 1985; Lillehoj, 1987b). Serum IgM peaked at 17 dpi of *E. tenella*(Trees et al., 1985). Serum IgG titers peaked at 17 dpi *E. tenella*(Trees et al., 1985). Although it is clear that coccidia-specific antibodies are abundant in serum following coccidiosis, the participation of these responses in protection against the parasites is unclear, as development is normally intestinal.

Macrophages act non-specially by phagocytizing and destroying antigens. They can also participate in the initiation of a specific immune response by processing the antigen and presenting it on their surface in association with MHC proteins to lymphocytes. Macrophages can also secrete cytotoxic compounds as well as cytokines that stimulate other immune cells. Cytokines are capable of stimulating macrophages to respond to *Eimeria* parasites. Perhaps an important T cell function is activation of macrophages. Immune serum enhanced macrophage phagocytosis of sporozoites of *E. tenella*(Onaga and Ishii, 1980). Thus macrophages may play a role in immunity, but may require activation by previous exposure to cytokines or the parasites.

Natural killer(NK) cells are usually considered to be a non-B non-T large granular lymphocyte(Ortaldo et al., 1979). NK cells act in a non-specific manner, killing foreign cells, virus-infected cells and tumor cells. Role of NK cells in the chicken intestine in coccidiosis has been studied(Lillehoj, 1989b). Following *E. acervulina* infection, splenic NK activity decreased at 2 dpi, then increased above that of

controls at 7 dpi(Lillehoj, 1989a). Splenic NK cell activity was also increased during secondary exposure to *Eimeria*(Lillehoj, 1989a). Since NK activity was assessed against an avian tumor line and not the parasites, the role these cells play is not clear. However, their increased killing activity could indicate a state of activation related to the presence of the parasites.

B) Mucosal responses to coccidia

Gut associated lymphoid tissue(GALT) is part of the systemic immune system, with similar features. There are a few features, however, that are unique to its function of providing protection against invasion and infection at mucosal surfaces. Both immunologically-mediated and non-immunological defenses play a role at the mucosal surface. These include normal mucosal flora, mucous secretion and movement via peristalsis or ciliary action and secretions of other substances such as lymph, gastric acid and bile salts.

Organized areas of lymphoid tissue in the vertebrate gut are present in peyer's patches(PP), and in chickens, in cecal tonsils and the bursa of Fabricius(BF). PP are complex structures primarily composed of lymphocytes, although antigen presenting cells(APC) such as macrophages and dendritic cells are also present. B cells are located in follicles, separated by interfollicular zones consisting mainly of T cells(Heyworth, 1988). BF contains B, T and macrophages but mainly serves as the site for B cell proliferation, differentiation and maturation in chickens. The cecal tonsils(CT), located near the ileocecal junction, contain organized lymphoid tissue which are B cell dependant follicular areas, interfollicular areas with T cells, and scattered macrophages. A majority of the lymphocytes are B cells expressing IgM(Lillehoj and chung, 1991). The term intraepithelial lymphocyte (IEL) is used to signify lymphocytes that occupy the intraepithelial regions of the gut. As in mammals, avian IEL are a heterogeneous population consisting of B cells, T cells, and NK cells(Lillehoj and Chung, 1991). The IEL of both mammals and birds appear to be mainly T cells, most of which bear the CD8 antigen. CD8+duodenal IEL were significantly decreased following primary *E. acervulina* infection and significantly increased after a secondary challenge infection(Lillehoj and Bacon, 1991). Thus cytotoxic T cells may play a role in resistance to secondary infections.

IEL exhibiting natural killer(NK) activity have been identified in both mammalian and avian species. NK activity was been reported predominantly in IEL and less in LP populations of chickens(Lillehoj, 1989a). Shortly following Eimerian infections, IEL NK activity was decreased, but in general was higher than that seen in uninfected chickens(Lillehoj, 1989a), suggesting that NK cells may be involved in parasite elimination. Activity of NK cells is generally increased by cytokines(Lillehoj, 1989b), again indicating the importance of T cell involvement by way of their stimulating effects on other cell populations. In addition to NK cells, the LP also contains B and T lymphocytes. Most of the lamina propria T cells bear the CD4 markers.

The immunoglobulin of major consequence in mucosal immunity is IgA. This is due to its high rate of synthesis and ability to be transported across the mucosal epithelium. However, IgM can also be transported across the epithelium. While circulating IgA is monomeric, secretory IgA(sIgA) is dimeric, or occasionally trimeric in mammals and usually trimeric or pentameric in chickens and turkeys. In addition to IgA

molecules, sIgA contains two other polypeptides, J chain and secretory component (SC). The J chain binding to IgA appears induces a conformational change that enhances polymerization(Crago and Tomasi, 1988). SC is a product of intestinal epithelial cells, hepatocytes and bile duct epithelial cells. The SC on the surface of these cells bind to polymeric but not monomeric IgA. The IgA-SC complex is internalized in endoplasmic vesicles, which move through the cell eventually fusing with the apical membrane, releasing sIgA into the lumen(Crago and Tomasi, 1988). SI_gA therefore enters the GI tract both by crossing the intestinal epithelium and in the bile.

Anti-coccidia sIgA was detected in bile and gut washings of coccidia-infected chickens (Lillehoj, 1987b; Rose et al., 1984a). Biliary sIgA was detected as early as 7 dpi *E. tentlla* or *E. acervulina*(Lillehoj, 1987b). Challenge infection with *E. tenella* or *E. acervulina* did not elicit an anamnestic sIgA response(Lillehoj, 1987b).

Antibodies may reduce invasion of some, but not all, species of *Eimeria* or may enhance intraluminal destruction of sporozoites. Immune sera have been shown to increase phagocytosis of sporozoites and merozoites by macrophage cultures(Onaga and Ishii, 1980). However, hormonal and/or chemical bursectomy did not affect the course of primary infection or secondary challenge(Giambrone et al., 1981; Lillehoj, 1987a) suggesting a minor role in protection against coccidiosis.

2) HOST GENETIC FACTORS INFLUENCING COCCIDIOSIS

The observations that certain strains within an animal species were differentially resistant to coccidiosis led to the investigation on the influences of host genetic factors on resistance or immunity to coccidiosis. The role of both major histocompatibility complex(MHC) associated genes and non-MHC genes has been studied. In chickens the *B*(Ea-*B*) system defines the MHC analogous to the H-2 system of mice and the HLA system in humans. The MHC codes for proteins involved in cell-cell communication and self/non-self discrimination. *B*-F genes encode Class I proteins, the self marker on all cells, *B*-L genes encode Class II proteins which are involved in cell-cell interactions and *B*-G genes encode an erythrocyte antigen for which there is no mammalian homologue. Non-MHC cellular antigen genes that may affect resistance to *Eimeria*(or disease in general) have been suggested at the A, C, D, E and I erythrocyte alloantigen loci(Johnson and Edgar, 1984 & 1986).

The availability of *B* congenic chickens, differing at the B complex but having the same background genes, has provided an opportunity to examine the effects of the MHC on resistance and immunity to coccidiosis. Additionally, chicken lines, sharing identical *B* haplotypes with various congenic lines but differing at other loci, have made it possible to study effects of non-MHC genes on infections. Studies on *B*-congenic and other chickens revealed that a wide variations in host responses exist (Lillehoj and Ruff, 1986; Bumstead and Millard, 1987; Lillehoj et al., 1989b; Lillehoj et al., 1990). Variations were seen in lesion scores, oocyst production, IgA levels, weight gain, and mortality although these measures did not always correlate with each other(Lillehoj et al., 1989b). In some cases, strains highly susceptible to primary infection were the most resistant to challenge infection, while other strains were susceptible to both primary and challenge infections(Lillehoj et al., 1989b) suggesting dif-

ferent genetic mechanisms controlling non-specific and specific immunity as well as immunological memory. SC(*B2*/*B2*) and FP (*B15*/*B21*) were compared in their immune response to coccidia, SC birds, which are more resistant than FP birds, showed substantially different immune responses compares to FP birds (Lillehoj and Ruff, 1986; Lillehoj et al., 1986). Furthermore, SC chickens showed higher levels of antigen specific antibodies early in infection, enhanced T cell responses to sporozoites and soluble *Eimeria* antigen and greater coccidial inhibitory activity mediated by PBL compared to FP chickens(Lillehoj et al., 1986). When SC chickens are compared with the 15.7-2(*B2*/*B2*) congenic line, the SC birds were more susceptible to primary infection but more resistant to challenge(Lillehoj et al., 1989b). The results of these studies seem to indicate that MHC and non-MHC genes are both capable of influencing the degree of resistance or susceptibility to Eimerian infections. Since genes influence both the immune system as well as non-immune factors which may affect the ability of a parasite to invade and multiply in a given host, it will undoubtedly require much additional research before genetic influences are more precisely understood.

3) BIOCHEMICAL CHARACTERIZATION OF COCCIDIAL ANTIGENS

Numerous coccidial antigens associated with the asexual and sexual stages of the coccidia life-cycle have been identified using polyclonal and monoclonal antibodies(mAbs). Many of these antigens have been molecularly cloned. In this section, the review of coccidial antigens will be divided into two categories, the asexual stage-associated and sexual stage-associated antigens.

A) Coccidia antigens associated with asexual stages of development

The complexity of coccidial antigens has been investigated by one- and two-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis(SDS-PAGE). Two-dimensional SDS-PAGE was performed to compare fingerprint maps of proteins from each of the 7 species of *Eimeria*(Sutton et al., 1989). Although 8 polypeptides were shared among all species, particular species could be identified by their unique fingerprint maps. In contrast, few detectable differences between strains of the same species were noted. Alterations in the polypeptides two-dimensional SDS-PAGE profiles were found when different developmental stages of the same species were examined.

Biosynthetic labeling of *E. tenella* sporozoites with ^{35}S-methionine followed by immunoprecipitation with immune chicken serum and one-dimensional SDS-PAGE revealed more than 3 labeled polypeptides with molecular weights ranging from <18,000 to >200,000(Wisher, 1986).

Size fractionation of sonicated sporozoite or oocyst extracts of *E. tenella* showed that most of the protective antigens were confined to a single pool of proteins in the molecular weight range of 20 to 30 Kda(Karkhanis et al., 1991). Further purification of the protective protein peak by gel filtration chromatography identified a 26 Kda polypeptide under reducing SDS-PAGE.

Coccidial antigens of second generation merozoite proteins are also complex. Sixteen to 22 total polypeptides were observed in the soluble antigen fraction of *E. tenella*, *E. acervulina*, and *E. necatrix*(Xie et al., 1992). However, the membrane fraction of all species had only 3

prominent protein bands, varying greatly in molecular weight among the different species. The immunogenicity of these proteins was investigated by their reactivity with *Eimeria* immune sera. The immunodominant antigens from merozoites of *E. acervulina*, *E. necatrix* and *E. tenella* cross-reacted with immune sera but unique bands were found indicating the presence of species-specific antigens.

Proteins synthesized by various subcellular organelles(for example, rhoptries, micronemes, and refractile bodies) of *Eimeria* have also been studied. Rhoptries are organelles commonly found in the motile, invasive stages of all coccidian parasites and are implicated in invasion of the host cell. It has been proposed that rhoptry proteins are enzymes, either proteases, phospholipases or phosphatases, that function by cleaving specific host cell components during the invasion process, thus playing a major function in events subsequent to parasite-host cell recognition and binding (Perkins, 1992). The contents of the rhoptry are discharged into the expanding vacuole after tight junction formation primarily to facilitate the formation of the parasitophorous vacuole membrane from the host membrane.

The rhoptry has a complex polypeptide profile with more than 30 different proteins identified. In *E. tenella*, 118, 122, 124, 132, 180, and 220 Kda proteins are prominent(Kazawoe et al., 1992) while 4 predominant rhoptry polypeptides of 63, 150, and 200 Kda were identified in *E. nieschulzi* (Dubremetz et al., 1989). Little information exists on the immunogenic relationship of rhoptry proteins between different stages of the *Eimeria* life-cycle. Most rhoptry polypeptides are recognized by sporozoite antisera but display only limited recognition by antisera merozoites suggesting that thy are almost exclusively sporozoite specific(Adam et al., 1990).

In contrast to rhoptry antigens, micronemes of *E. tenella* have a relatively simple polypeptide profile. Individual components of 24, 54, 94, 96, 100, and 110 Kda were identified(Kawazoe et al., 1992). The majority of microneme polypeptides are recognized by antisera to both sporozoites and second generation merozoites indicating considerable conservation of microneme epitopes between these stages.

Refractile bodies are the most conspicuous structure in sporozoites of most *Eimeria* species, occupying approximately 30~50% of the cytoplasmic area. Their function, however, is unknown. Antigens of the refractile body differ among the *Eimeria* species. Some antigens are conserved, whereas others differ in distribution among the individual species. Refractile bodies undergo rapid changes following invasion and may play a role in early development of the coccidia(Chobotar and Scholtyseck, 1982). Several protective antigens have been identified in the refractile body by molecular cloning. The recombinant GX3262 antigen is a β-galactosidase fusion protein containing a 112 amino acid *Eimeria* sequence with a predicted molecular weight of 12,000, smaller than the 28 Kda sporozoite refractile body protein identified by Western immunoblot analysis(Miller et al., 1989). The sequence of another protective recombinant antigen, CheYSO7', contains the sequence of GX3262(Crane et al., 1991).

Biochemical analysis of immunodominant surface antigens of *E. acervulina* sporozoites and merozoites revealed that 60% of sporozoite antigens and 90% of the merozoite antigens were observed to be ^{125}I surface labeled(Jenkins and Dame, 1987). Fifteen ^{125}I labeled sporozoite proteins with 7 predominant bands of 14~44 Kda were seen. Second and third generation *E.*

acervulina merozoites also displayed greater than 30 ^{125}I labeled proteins in the 15~35 Kda range with 11 predominant proteins of 20~85 Kda. When reacted with homologous immune serum, 10 immunodominant sporozoite antigens were identified ranging from 21 to 110 Kda in sporozoites and 20 to 250 Kda in merozoites.

B) Coccidial antigens associated with sexual stages of development

A number of gametocyte-associated antigens have been identified using mAbs. Two mAbs, T1A3B9(Laxer et al., 1987) and GD9(Larsen et al., 1991) that identify microgametocyte antigens of *E. tenella* associated with fertilization were characterized(Laxer et al., 1987). The mAb T1A3B9 reduced oocyst production by 50% in *in vitro* culture whereas the mAb GD9 showed a partial inhibition of oocyst production and lower lesion scores when given *per os* indicating an apparent inhibition of fertilization by blocking or preventing gamete attachment.

The immunogenic proteins of *E. maxima* gametocytes were investigated(Wallach et al., 1989). Two major gametocyte proteins of 56 and 82 Kda were recognized by immune serum obtained 14 days following infection. The major immunogenic proteins actively synthesized throughout late gametocyte development of *E. maxima* were examined by *in vitro* cell-free translation of mPNA(Mencher et al., 1989). They ranged from 50 to 225 Kda with the major immunogenic components being 50, 65, 95, 100, and 225 Kda. The *in vivo* role of these antigens should be further tested to determine their importance in parasite growth and development. These studies indicate that epitopes present on these protein antigens could be used for development of a vaccine against the sexual stages of *Eimeria* to reduce or prevent disease transmission.

4) VACCINE DEVELOPMENT

Numerous vaccination strategies have been attempted to control avian coccidiosis. A mixture of live parasites has been successfully used in commercial application, but due to potential problems associated with using live parasites, various means of attenuating *Eimeria* pathogenicity have been tried. Attenuation has been achieved by embryo adaptation, selection of precocious strains, X-irradiation, and chemical treatment. Selection of precocious strains of coccidia has produced the best results. All 7 species of precocious coccidia are currently being used as commercial vaccines in Europe. Because of the need to develop a nonreplicating coccidian vaccine, many laboratories have focused their efforts using recombinant DNA technology. Many potentially protective coccidian antigens have been genetically engineered and used in experimental immunizations. However, the identification of a recombinant coccidial vaccine has been difficult due to the complex parasite life-cycle, incomplete understanding of the mechanisms of protective immunity, and species specificity of immunity seen in natural infections.

A) Live virulent vaccines

A live coccidiosis vaccine consisting of all species of virulent coccidia was introduced in 1964(Edgar). The efficacy of live parasite vaccination is influenced by the mode of administration. Continuous exposure with a low number of oocysts(5/day) induced significantly greater immunity compared to a single immunization with the same number of oocysts (Joyner and Norton, 1973). Oocyst production in repeatedly infected birds extended well beyond

the period of patency of a single infection, but eventually ceased and the birds appeared to be highly immune compared to a single immunization regimen. The live vaccine, however, is mostly limited to breeder flocks and is not suitable for broiler production because of potential accumulation of live parasites in the litter. Attenuated live vaccines have been used in the broiler industry for the last 10 years(Bushell et al., 1989).

B) Live attenuated vaccines

Attenuating the pathogenicity of coccidia is accomplished by *in vivo* embryo passage(Long, 1972, 1974), selection of precocious strains (Jeffers, 1975), x-irradiation(Hayat, 1976) and microwave treatment.

The chicken embryo-adapted H strain shows an absence of large second-generation schizonts normally found in the tunica propria of the cecum. An embryo-adapted line of *E. necatrix* shows similar attenuation but with lower reproductive potential and markedly less immunogenicity than its precocious line(Shirley and Bellatti, 1984). Chickens kept in litter pens and given small numbers of oocysts of embryo-adapted coccidia were substantially immune(McDonald et al., 1986).

Precocious selection is accomplished faster and yields more stable strains of attenuated coccidia than embryo selection(Jeffers, 1975). The general method utilizes repeated passage of virulent coccidia, each time selecting for early produced oocysts. The evidence that selection of precocious strains results in attenuation of pathogenicity was first reported by Jeffers (1974) and subsequently extend to all species of *Eimeria*. The wild-type life-cycle normally consists of 3 or 4 generations of the schizogony followed by gametogony and subsequent oocyst excretion. Precocious strains, in contrast, typically undergo only 2 or 3 generations of schizogony due to deletion or substantial depletion of the final generation. Such changes to the normal endogenous life-cycle are accompanied by marked reductions in prepatent time, multiplication rate, and pathogenicity. Their immunogenic properties, however, remain equivalent to the parental parasites.

The immunogenicities of precocious strains of all 7 *Eimeria* spp. were verified by Shirley and Millard(1986). Each strain was characterized by an abbreviated life-cycle in which one generation of schizont was either substantially missing(*E. acervulina* and *E. mitis*) or reduced in size (*E. tenella*). Substantial protective immunity was also shown using oocysts of precocious lines of six *Eimeria* species delivered in alginate beads following challenge with virulent strains (Johnson et al., 1986).

Two characteristics of precocious strains have practical implications for their large-scale commercial use. First, despite their reduced reproductive potential, it is possible to obtain a large number of oocysts from them. Second, precocious strains are stable in the field. However, some precocious strains of coccidia may not elicit complete immunity to infection. Furthermore, this vaccine will not engender protection with heterologous parasites or parasites showing antigenic diversity.

C) Recombinant antigen vaccination

Concerns have been raised about the stability, quality control, cost-effectiveness, and efficacy of live or precocious vaccines against the vast array of field strains likely to be encountered in vaccination programs in different geographical areas(Chapman, 1988). Thus, the feasibility of using recombinant coccidial vaccines, which ad-

dress some of these concerns, has been tested. A large number of cDNAs encoding *Eimeria* antigens have been described(Profous-Julchelka et al., 1988; Jenkins et al., 1988 and Miller et al., 1989) and immunization trials using these antigens are underway. It is important to emphasize a variety of factors that must be considered when developing a recombinant *Eimeria* vaccine. Among these are host genetic background and mode of antigen delivery. When various chicken lines of defined genetic constitution were immunized with the recombinant cSZ-1 or cMZ-8 proteins of *E. acervulina*, differences in the development of resistance were demonstrated following experimental challenge infections(Lillehoj et al., 1988b; Lillehoj et al., 1990).

With regards to the mode of immunization, administration of the cMZ-8 antigen conferred partial protection when given in adjuvant(Miller et al., 1989) or in live *E. coli*(Kim et al., 1989; Miller et al., 1989) against challenge infection with live coccidia. Chickens immunized parenterally with the cMZ-8 protein showed higher antibody titers and T-cell responses compared to chickens inoculated orally(Lillehoj, 1991). Oral immunization of chickens with live *E. coli* transformants carrying the cMZ-8 gene was more effective in the induction of local immunity in some strain of chickens whereas parenteral immunization was more effective in other strains. Both cellular and humoral antingen specific responses were observed following immunization of chickens with live *E. coli* transformants(Kim et al., 1989).

A variety of other recombinant antigens potentially useful for coccidia vaccine development have been reported. The recombinant antigen GX3262, also produced as a β-galactosidase fusion protein by *E. coli*, was identified in a cDNA library of *E. tenella* using polyclonal hyperimmune chicken serum(Miller et al., 1989). GX3262 was also reactive with the monoclonal antibody MCA 12-09, which detects a cross-reactive sporozoite antigen of all 7 *Eimeria* species. To study the immunogenicity of the GX3262 antigen, broiler chickens were immunized with partially purified GX3262, heat-killed recombinant *E. coli* expressing GX3262, or with live recombinant *E. coli* expressing GX3262, All antigens were adsorbed onto alhydrogel adjuvant. A single subcutaneous inoculation of 2 day-old broilers with 25 μg of GX3262, administered in the form of a heat-inactivated bacterin induced partial protection against *Eimeria* infection. Three subcutaneous injections with partially purified recombinant GX3262 at weekly intervals induced a high level of protection against coccidiosis. Boosting of GX3262 immunized chickens with 25 live *E. tenella* oocysts enhanced the immunity generated by the recombinant antigen.

The results of a recent study suggest the possibility of using the recombinant GX3262 antigen to induce cross-species immunity against multiple *Eimeria* spp.. When GX3262 immune chickens were given a small number of *E. tenella* or *E. acervulina* oocysts, enhanced protection against *E. tenella* or *E. acervulina* experimental challenge was observed(Bhogal et al., 1992). Furthermore, a booster inoculation with 200 *E. acervulina* oocysts, 8 days after vaccination with GX3262 bacterin, enhanced immunity against *E. tenella* infection. Chickens immunized with live recombinant *E. coli* expressing GX3262 were protected against both *E. tenella* and *E. acervulina* infection. In another study, chickens immunized with the recombinant CheYSO7' antigen were protected against 4 different

Eimeria species(Crane et al., 1991). The *E. tenella* fusion protein CheYSO7' is expressed in *E. coli* as a 36 kDa protein and identified using chicken antisera(Karkhanis et al., 1991). Western immunoblot analysis of oocysts from 7 different *Eimeria* species with rabbit CheYSO7' antiserum revealed a prominent band at 26 to 28 kDa. Because this antigen was identified in all 7 species, it is possible that it could confer cross-protection against multiple coccidia species. Although chickens immunized to natural infection develop antibodies to this protein, active immunization of 2 to 4 day-old chickens with a protective dose of CheYSO7' without adjuvant did not elicit a humoral rrsponse suggesting that the partial protection is the result of cell-mediated immune effector mechanisms.

PERSPECTIVES

Development of an efficient coccidiosis vaccine has been slow. Partial protection can be induced in young chickens by vaccination with non-living parasite antigens or recombinant antigens. The particular stage of the parasite life-cycle important in inducing protection is generally thought to be an early asexual stage. Two compelling observations support this contention : 1) strong immunity provided by a immunization with WIS-F-96, a precocious strain missing a second generation meront stage, and 2) lack of immunity by parasites inhibited to develop beyond the sporozoite stage. Induction of protective immunity by active immunization with antigens not involved in natural infection may also be possible as indicated by recent immunization studies using recombinant antigens. In this regard, any recombinant antigen derived from a particular stage of the *Eimeria* life-cycle would most likely be combined with other stage specific antigens to increase the vaccine's potential.

Although the protection induced by recombinant antigens is real, it may not be of sufficient potency to compete with prophylactic chemotherapy now in standard practice. Other vaccine strategies have thus been tried, for example use of synthetic peptide based vaccines. *In ovo* delivery represents another novel alternative. The ultimate development of an efficacious vaccine against coccidiosis awaits continued research and development efforts directed toward these various strategies.

ABSTRACT

Coccidiosis is caused by *Eimeria* infecting primarily the intestine of the susceptible host, thereby seriously impairing the growth and feed utilization of livestock and poultry. The genus *Eimeria* contains a number of obligate intracellular protozoan parasites with a complicated life-cycle involving both asexual and sexual stages of development. The desire to develop a vaccine against *Eimeria* has promoted active research to elucidate the mechanisms of protective immunity and identification of candidate vaccine antigens. Protozoa are unique in their modes of transmission and nature of disease manifestations, the significance of which should be considered in the development of a control strategy. An intricate and complex interplay of different cell populations and cytokines is involved not only in the pathogenesis of coccidiosis but also in the development of protective immunity. Thus, comprehensive understanding of the events leading to protection following *Eimeria* infection will be crucial for the development of an effective vaccine.

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