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Cellular and Molecular Identification of *Eimeria acervulina* Merozoite Antigens Eliciting Protective Immunity

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CELLULAR AND MOLECULAR IDENTIFICATION OF EIMERIA ACERVULINA
MEROZOITE ANTIGENS ELICITING PROTECTIVE IMMUNITY

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Abstract

Coccidiosis, ubiquitous diseases of poultry, seriously impair the growth and feed utilization of livestock and poultry. Coccidiosis causes over \$600 million annual losses world-wide and no vaccine is currently available. The goal of this study was to investigate the cellular and molecular mechanisms controlling protective immune responses to coccidia parasites in order to develop immunological control strategy against coccidiosis. The major findings of this study were: 1) cell-mediated immunity plays a major role in protection against coccidiosis, 2) when different genetic lines showing different levels of disease susceptibility were compared, higher T-cell response was seen in the strains of chickens showing higher disease resistance, 3) early interferon secretion was observed in more coccidia-resistant chicken strains, 4) both sporozoite and merozoite antigens were able to induce interferon production, and 5) chicken monoclonal antibodies which detect immunogenic coccidia proteins have been developed. This study provided a good background work for future studies toward the development of recombinant coccidial vaccine. Availability of chicken monoclonal antibodies which detect immunogenic coccidia proteins will enhance our ability to identify potential coccidial vaccine antigens.

B. DETAILED DESCRIPTION OF THE RESEARCH ACTIVITIES AND RESULTS

1. Elucidation of the cellular mechanism(s) influencing genetically controlled disease resistance.

Previous studies from this laboratory demonstrated that cell-mediated immunity plays a major role in host protection against avian coccidiosis. In order to better characterize the underlying immune mechanisms which control genetically determined disease resistance and susceptibility to coccidiosis, we have performed series of studies to compare host immune responses to coccidia parasites in several chicken strains which show different levels of disease susceptibility to coccidiosis. Our previous report included results from our initial study comparing mitogen-induced T-cell proliferation among several chicken lines congenic at the B-locus, the major histocompatibility complex of chickens (Martin et al., 1993). The results showed that these MHC-congenic chickens demonstrate differing response to mitogen-induced T-cell proliferation and mitogen-induced interferon production following infection with Eimeria acervulina. In view of recent studies showing that various cytokines immunoregulate host immune response to parasites, we have examined the role of macrophage-secreted products in E. tenella infection. Our earlier work showed that following E. tenella infection, high level of tumor necrosis-like factor (TNLF) was detected in serum. To further investigate the role of macrophage-secreted product in avian coccidiosis, we treated chickens with either crude TNLF or rabbit anti-TNF sera

to neutralize endogenous production of TNLF following E. tenella infection. The results showed that SC strain of chickens, which are less susceptible to E. tenella infection compared to TK strain of chickens, showed higher level of TNLF production than TK chickens. Furthermore, neutralization of endogenous TNLF in E. tenella-infected SC chickens resulted in improvement in weight gain. These results indicate that macrophages mediate protective immunity against coccidiosis via a soluble factor-dependant mechanism and TNFL may be involved in E. tenella-induced weight loss. Collectively these studies demonstrate importance cell-mediated immunity mediated by various lymphocytes and macrophages.

Meat-type broiler chickens which differ respect to the MHC class IV genes were used to investigate the role of Class IV MHC gene in E. acervulina infection (Uni et al., 1995). When intestinal lesions and plasma carotene concentrations were used to evaluate differences in disease susceptibility to E. acervulina infection, Chickens with KK and FK class IV MHC haplotypes were more resistant than AA, FF and FA chickens. The results showed that a possible association exists between susceptibility to E. acervulina infection and disease susceptibility to E. acervulina infection.

2. IDENTIFICATION AND PHENOTYPIC CHARACTERIZATION OF THE SUBPOPULATION OF T CELLS MEDIATING PROTECTION

Last year we reported that cytotoxic T-lymphocytes expressing CD8 antigen are responsible for protection against coccidiosis. To better characterize the nature of

intestinal lymphocytes mediating protective immunity, chickens were treated with various monoclonal antibodies detecting chicken lymphocyte subpopulations. The results showed that chickens treated with the monoclonal antibody which detect CD8+ lymphocytes, but not CD4+ lymphocytes, showed severe infection following secondary infection with E. acervulina. In summary these studies provide a strong evidence that cytotoxic T-cells play a critical role in host defense against Eimeria parasites.

3. IDENTIFICATION AND MOLECULAR CLONING OF PROTECTIVE ANTIGENS OF E. ACERVULINA

Identification and characterization of immunogenic coccidial antigens eliciting cell mediated as well as humoral immune responses are vital to effective vaccine development. Since the results of our study indicate that T lymphocytes mediate protective immune response to coccidiosis, our initial goal was to identify coccidial antigens recognized by coccidia-specific T cell clones. Development of T-cell clones specific for coccidia proteins were tried last year without much success due to our inability to clone chicken IL-2 gene which encodes a T-cell growth factor. To identify IL-2 gene in chickens, we used mouse, human and bovine cDNA encoding IL-2 in northern hybridization. These studies did not produce any positive clones presumable due to a evolutionary distance between birds and mammals. Since we were not successful in cloning chicken IL-2 gene, we tried to grow coccidia-specific T-cells in the presence of crude conditioned medium from concanavalin A-stimulated spleen cell

culture. In these trials, we could maintain the growth of coccidia-specific cells for up to 40-50 days but then they failed to grow. Therefore, other approaches toward the identification of potential coccidial vaccine antigens were attempted. One of this approach was to develop chicken hybridomas which can identify important coccidial antigens (Lillehoj et al., 1994. Poultry Science 73). The reason for choosing this system was that mouse monoclonal antibodies may not detect epitopes of coccidia parasites which are relevant for chicken system. We have now several stable chicken hybridomas which secrete monoclonal antibodies detecting sporozoite and merozoite antigens of Eimeria. These antibodies are currently used to identify immunogenic coccidia genes which can be potential vaccine antigens.

C. EVALUATION OF THE RESEARCH ACHIEVEMENTS WITH RESPECT TO THE ORIGINAL RESEARCH PROPOSAL AND ITS OBJECTIVES

The studies described in this report closely relate to our original objectives as stated in the BARD Grant US-2047-91R. The studies supported by the BARD Grant enhanced our understanding of underlying host immune mechanisms which are critical in host resistance to avian coccidiosis. Furthermore, novel strategy of chicken hybridoma development for the first time for identifying immunogenic coccidia antigens which are relevant to chickens was developed and availability of chicken anti-coccidia monoclonal antibodies are currently being used to identify various coccidial genes which can be potential vaccine.

D. DESCRIPTION OF COOPERATION

Dr. Heller visited Dr. Lillehoj's laboratory for 1 year to molecular clone coccidial cDNAs and to develop in vitro assays to assess cell-mediated immunity. Dr. Lillehoj provided Dr. Heller live culture of E. acervulina and mouse monoclonal antibodies specific for CD4 (helper T cells), CD8 (cytotoxic T cells), CD3 (pan-T lymphocytes) and K1 (macrophages) antigens that were developed in Beltsville. Dr. Heller will use these monoclonal antibodies to evaluate host immune responses following coccidial infections in broiler and leghorn chickens maintained at the Hebrew University.

E. CONCLUSIONS

Avian coccidiosis results in the extensive destruction of the intestinal epithelium accompanied by severe loss in body weight, reduced feed efficiency, and eventual mortality in chickens. As a result, coccidiosis is estimated to cost the US poultry industry over \$600 million annually. An effective vaccine against coccidiosis does not currently exist. Understanding the immunobiology of host-parasite interactions leading to parasite elimination in the gut is crucial for the design of an effective vaccine.

The main focus of this study was the elucidation of the mechanisms of protective immunity and identification of protective antigens of Eimeria. The results indicate that an intricate and complex interplay of different cell populations and cytokines not only are involved in the pathogenesis of the infections but also development of protective immunity. The results of the studies conducted in this proposal collectively indicate

a role for T lymphocytes in protection against coccidiosis and provided necessary information for recombinant vaccine development for avian coccidiosis. Furthermore, availability of stable monoclonal antibodies detecting immunogenic proteins of coccidian parasites will now enable us to identify potential vaccine antigens of Eimeria.

F. LIST OF PUBLICATION

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2. H. S. Lillehoj, K. Sasai and H. Matsuda. 1994. "Development and characterization of chicken-chicken B cell hybridomas secreting monoclonal antibodies that detect sporozoite and merozoite antigens of Eimeria. Poultry Science 73:1685-1693.
3. A. M. Martin, S. Awadalla and H. Lillehoj. 1994. Enhanced humoral and cell mediated responses to eimeria acervulina following inoculations with merozoite and sporozoite fractions. Beltsville Agricultural Research Center Symposium.
4. K. Sasai, H. S. Lillehoj, H. Matsuda, T. Miyamoto, T. Fukata, E. Baba and A. Arakawa. Generation of chicken-chicken monoclonal antibody against Eimeria acervulina antigen associated with CD8+ T cell:Effect on ultrastructure using immunofluorescent scanning microscopy and cellular invasion. 9th Japanese-German Cooperative Symposium on Protozoan Diseases. Hokkaido, Japan.
5. Z. Uni, D. Sklan, N. Haklay, N. Yonash and D. Heller. 1995. "Response of three class-IV major histocompatibility complex haplotypes to Eimeria acervulina in meat-type chickens". British Poultry Science. 36:555-561.
6. A. M. Martin, S. Awadalla and H. Lillehoj. 1995. "Characterization of cell mediated responses to Eimeria acervulina antigens. Avian Diseases 39:538-547.