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BARD

FINAL REPORT

PROJECT NO. IS-3021-98

**Molecular Genetic Dissection of Complex
Immunocompetence Traits in Broilers**

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Project Title:

Molecular Genetic Dissection of Complex Immunocompetence Traits in Broilers

Investigators

Institutions

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Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Antibody; candidate genes; DNA markers; *Escherichia coli*; microsatellites; major histocompatibility complex; quantitative trait locus; *Salmonella enteritidis*; selection; single nucleotide polymorphism; spleen; vaccine;

Abbreviations commonly used in the report, in alphabetical order:

Ab – antibody; DNA - deoxyribonucleic acid; EC - *Escherichia coli*;
ELISA - enzyme-linked absorbant assay; HH - broiler line selected for high EC antibody;
LL - broiler line selected for low EC antibody; MDV - Marek's disease virus;
MHC - major histocompatibility complex; PCR - polymerase chain reaction;
QTL - quantitative trait locus (loci); SE - *Salmonella enteritidis*;
SNP - single nucleotide polymorphism

Budget: IS: \$128,000

US: \$137,000

Total: \$265,000

A. Cahaner

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)	2	10	8	20
Submitted, in review, in preparation	-	-	2	2
Invited review papers	-	-	-	-
Book chapters	1	1	-	2
Books	-	-	-	-
Master theses	-	-	-	-
Ph.D. theses	-	1	2	3
Abstracts	-	12	4	16
Not refereed (proceedings, reports, etc.)	-	3	-	3

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	1	1	3	5
Longer Visits (Sabbaticals)	-	-	-	-

Description of Cooperation:

Patent Summary (numbers)

	Israeli inventor (s) only	US inventor (s) only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

Abstract

Objectives:

(1) Evaluate Immunocompetence-QTL-containing Chromosomal Regions (ICRs), marked by microsatellites or candidate genes, for magnitude of direct effect and for contribution to relationships among multiple immunocompetence, disease-resistance, and growth traits, in order to estimate epistatic and pleiotropic effects and to predict the potential breeding applications of such markers.

(2) Evaluate the interaction of the ICRs with genetic backgrounds from multiple sources and of multiple levels of genetic variation, in order to predict the general applicability of molecular genetic markers across widely varied populations.

Background: Diseases cause substantial economic losses to animal producers. Emerging pathogens, vaccine failures and intense management systems increase the impact of diseases on animal production. Moreover, zoonotic pathogens are a threat to human food safety when microbiological contamination of animal products occurs. Consumers are increasingly concerned about drug residues and antibiotic-resistant pathogens derived from animal products. The project used contemporary scientific technologies to investigate the genetics of chicken resistance to infectious disease. Genetic enhancement of the innate resistance of chicken populations provides a sustainable and ecologically sound approach to reduce microbial loads in agricultural populations. In turn, animals will be produced more efficiently with less need for drug treatment and will pose less of a potential food-safety hazard.

Major achievements, conclusions and implications: The PI and co-PIs had developed a refined research plan, aiming at the original but more focused objectives, that could be well-accomplished with the reduced awarded support. The successful conduct of that research over the past four years has yielded substantial new information about the genes and genetic markers that are associated with response to two important poultry pathogens, *Salmonella enteritidis* (SE) and *Escherichia coli* (EC), about variation of immunocompetence genes in poultry, about relationships of traits of immune response and production, and about interaction of genes with environment and with other genes and genetic background. The current BARD work has generated a base of knowledge and expertise regarding the genetic variation underlying the traits of immunocompetence and disease resistance. In addition, unique genetic resource populations of chickens have been established in the course of the current project, and they are essential for continued projects.

The US laboratory has made considerable progress in studies of the genetics of resistance to SE. Microsatellite-marked chromosomal regions and several specific genes were linked to SE vaccine response or bacterial burden and the important phenomenon of gene interaction was identified in this system. In total, these studies demonstrate the role of genetics in SE response, the utility of the existing resource population, and the expertise of the research group in conducting such experiments.

The Israeli laboratories had showed that the lines developed by selection for high or low level of antibody (Ab) response to EC differ similarly in Ab response to several other viral and bacterial pathogens, indicating the existence of a genetic control of general capacity of Ab response in young broilers. It was also found that the low-Ab line has developed, possibly via compensatory "natural" selection, higher cellular immune response. At the DNA levels, markers supposedly linked to immune response were identified, as well as SNP in the MHC, a candidate gene responsible for genetic differences in immunocompetence of chickens.

Achievements

Salmonella enteritidis (US part)

Antibody and pathogen load genetic associations

An important finding is that the genetic potential to produce antibody to SE vaccine, measured in full-sibs of non-vaccinated chicks that were challenged with pathogenic SE, is associated with pathogen load. This resolves the apparent lack of association between antibody levels and bacterial response that are often measure when antibodies are directly assayed on the pathogen-challenged birds.

Candidate gene associations

A major focus of the SE portion of the project has been the examination of candidate genes. Due to the extensive literature on genes and pathways in the mechanisms of intracellular bacteria in mice, this has been a very successful approach. The following genes, or the chromosomal region containing them, were linked to SE challenge or vaccine response: MHC, Nramp1, inhibitor of Apoptosis-1, Caspase-1, Prosaposin, Inducible nitric oxide synthase, Transforming growth factor beta 3 and 3, tumor necrosis factor related apoptosis inducing ligand, and immunoglobulin light chain.

Selection on Ab response to *Escherichia coli* (Israel part)

Multi differences in immune response traits between the selection lines

HH birds exhibited higher titers of Ab Marek's disease virus (MDV) than LL chicks at 18 days of age, and also at 4 and 7 months of age. F₁ birds exhibited intermediate titers, indicating additive genetic control of Ab response. Positive phenotypic correlation was found between Ab responses to EC and to MDV within each line. Very high positive genetic correlations were found between means of Ab following EC exposure and Ab response to vaccines of infectious bursa disease virus (IBDV), Newcastle disease virus (NDV), MDV, and SE. The results indicate that the divergent selection on Ab response to EC had led to a genetic divergence between the lines in genes controlling the general ability of young broilers to produce Ab.

The frequency of T lymphocytes bearing the CD8 receptor (CD8⁺ cells) in peripheral blood and spleen was higher in LL chicks than HH chicks. Higher % of CD8⁺ cells, which play a role in cell-mediated responses, suggest that LL chicks are superior to HH chicks in this immune system, compensating for their lower humeral response. The genetic basis of this difference in cell-mediated immune response appears to be independent of the that of Ab response, suggesting that simultaneous genetic enhancement of both immune responses might be feasible.

Evaluation of DNA markers' effect on various immune traits

Seven DNA markers were significantly associated with at least one trait: ADL146 with body weight; ADL160 with %mortality; and the other five markers were associated with Ab responses: MCW183, MCW83, MCW234 and LEI104 with Ab response to IBDV, and LEI104 also with Ab response to EC vaccine. The effects of MCW234 and LEI104 were repeated in several F2 families. Moreover, the genotypes of these markers were associated also to other immune traits and body weight.

MHC class I DNA sequence polymorphism among the HH and LL lines

A set of two primers (designed by Lamont) were used to amplify the $\alpha 2$ domain of the chicken MHC class I gene, representing part of the variable peptide binding cleft. The PCR products were sequenced, and sequence alignments within line and between lines allowed to determine single nucleotide polymorphism (SNP) at this region, which was higher in LL than in HH chicks. No SNP was line-specific, in agreement with the genetic variation in Ab or cellular responses, found within the selection lines. However, in six of the 19 SNP positions, the lines differed significantly in the SNP variants, leading to differences in the structure of the $\alpha 2$ domain of the MHC class I receptor at two amino-acid positions, located at the antigen-binding cleft. Changes in the MHC class I receptor, especially at the cleft area, can affect its ability to present antigens, hence they may explain immune response differences between the lines.

Cooperation: There had been intensive scientific exchanges between the US and Israeli investigators. At the onset of the project, the Co-PI (Lamont) visited Israel and met the local PIs. By the end of 1st year, the Israeli PI (Cahaner) and two PhD students who had worked on this project (Yunis and Lavi) met with Lamont during a conference in Canada; technical details and findings on both sides were thoroughly discussed. Following this meeting, Lamont provided primers of an MHC gene that were utilized by the Israeli team to search for SNP in the Israeli EC-selection lines. This part was done in close collaboration with Dr. J. Pitcovski, an Israeli expert in molecular immunogenetics. By the end of 2nd year, Cahaner and Lamont met during a symposium in Turkey. They discussed the 2nd annual report, the work to be done during 3rd year, and plans for continuation. In view of the highly important SNP results in candidate genes, it was decided to continued at this direction, with Pitcovski taking the lead in Israel. Pitcovski visited Lamont in July 2002 to prepare a continuation proposal, which later won BARD support. Cahaner and Lamont met again in September 2002 in Germany, and discussed the final phase of the project.

Grant-Related Publications for Entire Period of BARD Grant (1999-2003):

Refereed Journal Papers:

- Kaiser, M. G., N. Deeb, and S. J. Lamont. 2002. Microsatellite markers linked to *Salmonella enterica* serovar enteritidis vaccine response in young F₁ broiler-cross chicks. *Poultry Sci.* 81:193-201.
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Presentations Published in Symposium Proceedings:

Lamont, S. J. 2002. Candidate genes for immune response and disease resistance in chickens. Proceedings of the National Breeder's Roundtable Meeting. St. Louis, MO. Poultry Breeders of America, Decatur, GA.

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