OFFICIAL

REGIONAL PROJECT OUTLINE

PROJECT NUMBER: NE-60 (Rev.)

TITLE: Genetic Bases for Resistance and Immunity to Avian Diseases

DURATION: October 1, 1998 - September 30, 2003

STATEMENT OF THE PROBLEM

Disease losses represent a significant component in the overall cost of poultry production. The sequelae of specific diseases produce mortality as well as lower performance either due to overt or subclinical conditions. In some cases, more virulent pathogens have arisen which are less responsive to current vaccines or therapies. Understanding the genetic bases for disease resistance and immunity will lead to more effective prevention and treatment procedures. These new methods will increase production efficiency and lower costs.

JUSTIFICATION

World-wide consumption of poultry products has increased drastically during the last 25 years. The total number of chickens produced including layers in 1993 was 12,009 x 10^6 world-wide and 1,529 x 10^6 in the USA (FAO Animal Health Yearbook 1993). These figures are an underestimation of the real production in the USA and by implication for the world. The USDA Poultry Yearbook (1996) indicates that 6,700 x 10^6 broilers were produced in the USA in 1993. The production for 1996 was estimated at 7,600 x 10^6 broilers and 300 x 10^6 layers for the USA alone. Although the number of layers has remained stable over the last 16 years, the number of broilers has doubled during the same period. The total gross income of broiler and egg production in the U.S.A represents a value of over \$18,664 x 10^6, which does not include the added value for allied industries. The total export value of poultry products for 1991 was \$1.1 billion, which makes the USA the leading export nation of the world, followed by France and Brazil (Poultry Times, XXXIX, No. 11, May 25, 1992). This value has increased during the last five years mostly due to increased export of broilers. Broiler exports in 1996 were 2.07 x 10^6 metric tons with a value of \$2,027 x 10^6, (Broiler Industry 60 (5):22, May, 1997) which represented a 20.8% increase over 1995. Further growth in the production of broilers is expected based on a shift in consumer preference towards poultry meat instead of red meat, and the increased needs of the former Soviet Union and Eastern Europe which are potentially important export markets for the USA.

Total losses caused by specific diseases include not only mortality, lower egg production, and condemnations but also costs of vaccination, chemotherapy, and eradication programs. Although there are no recent published data available on these losses, Biggs (1982) quoted a loss of \$1,427 x 106 in 1975 for the USA alone. Calnek and Witter (1997) estimated that the total losses caused by Marek's disease (MD) [mortality, vaccination costs, reduced egg production] were close to one billion dollars worldwide in 1984. These losses have certainly increased over the last 17 years, especially in view of the emergence of ever more virulent strains of MDV. These so-called vv+MDV strains are causing outbreaks of MD in chickens properly vaccinated with MDV (Witter, 1997). The recent problems with *Salmonella enteriditis* have caused considerable economic losses and further losses can be expected. For example, if FDA suggestions for the elimination of *S. enteriditis* are implemented, the costs of table eggs will

increase dramatically (Dr. D. Kradel, personal communication). In the USA, the losses caused by *S. enteriditis* are based on the impact on human health of only a small number of positive hens. In addition to the clearly identifiable problems, a substantial portion of the losses are caused by suboptimal production as a consequence of interactions among management, genetic resistance and disease agents (Biggs, 1982).

Reduction of these losses depends on several interrelated factors; the interaction between genetic background of the chicken and the development of the immune responsiveness is especially relevant. This will become even more important with the advent of biotechnology applications. The **ARS-ADOL** NE-60 station has preliminary evidence that certain genetic strains exhibit diverse responses to different MD vaccines. Future recombinant vaccine development and use will proceed with the ability to tailor the vaccines to specific genetic strains. However, manufacturing these tailored vaccines will require a better understanding of humoral and cell-mediated immune (CMI) responses. Recently developed techniques to study the role of specific gene products in the generation of efficient CMI responses will allow the selection of important genes from MDV and other avian viruses for insertion in recombinant vaccines.

Immune responsiveness is at least in part determined by the major histocompatibility complex (MHC) as well as by other genetic traits. The collaborating stations of NE-60 have demonstrated MHC-related resistance to a number of diseases. Information demonstrating that genetic selection related to immunity may reduce economical losses is important, because successful adaptation of appropriate selection procedures by primary breeders may lead to a rapid dissemination of more resistant strains and a subsequent reduction in losses. NE-60 has invited representatives of breeder organizations to the annual meetings to disseminate new information on an informal basis. These breeder representatives also bring information on field problems and comments on the importance of approaches taken by the Committee.

Two previous five-year project outlines stated that rapid progress in the understanding of the interactions between genetic background and disease resistance was expected over the next 5 to 10 years. This prediction was based, in part, on the development of MHC-congenic strains and the development of monoclonal antibodies (Mabs) for lymphocyte (sub)populations by several participating stations of NE-60. Both efforts have indeed provided exceptionally valuable tools for our research, which is reflected in the 202 publications from the project including a substantial number of joint publications among NE-60 stations (Publications list).

In addition, many stations have become involved in the direct application of biotechnology in their research efforts. It is expected that NE-60 stations will be leaders in the isolation of important genes for the immune responses in chickens. These developments will be of interest to the regional project NC-168 (Advanced technologies for the genetic improvement of poultry). Collaborative efforts between both technical committees are certainly expected, in which transgenic chickens developed by NC-168 can be used by NE-60 for the evaluation of transgenic genes for immunological functions and disease resistance. Likewise, NE-60 may be able to identify important genes which can be mapped by NRSP-8 and utilized by NC-168 in strategies for marker assisted selection as well as for the generation of transgenic chickens.

The proposed studies will require the application of advanced techniques in immunology, biochemistry, virology, bacteriology, parasitology, molecular biology and genetics to study pathogens using well-defined strains of chickens in facilities designed to contain infectious agents. NE-60 members form the ideal team to pursue the proposed studies, because they have: i) a proven collaborative research record, and ii) as a group the needed expertise listed above.

The group's critical knowledge is clearly illustrated in the two recently published avian immunology books (Davison *et al.*, 1995; Davison *et al.*, 1996). Project members or collaborators made substantial contributions to both books that demonstrate the productivity and standing of this group.

RELATED CURRENT AND PREVIOUS WORK

Extensive searches of publications for the last five years indexed in the Agricola, Biosis, CAB (British), CRIS, Health Index, and Medline comprehensive databases revealed that NE-60 members have made substantial contributions to scientific progress in genetics of disease resistance and immune response in poultry. Many publications are joint-authored between participating stations because of the multidisciplinary nature of the problems addressed. This fact illustrates the truly essential, cooperative nature of the project. Other groups outside the U.S. conduct research complimentary to that of NE-60. Research on the chicken MHC is conducted at stations in Australia, Czech Republic, Denmark, France, Israel, the Netherlands, and the U.K. Selection on non-MHC traits is also conducted in the Netherlands, France, and Israel. Marek's disease research programs exist in Japan and the Netherlands. In most instances, either formal or informal working relationships exist between NE-60 stations and the international laboratories conducting similar research. This assures coordination of efforts and avoidance of unnecessary duplication of research.

It is relevant to consider the relationship of NE-60 to other regional projects addressing problems in poultry genetics or disease in the U.S. NE-138 "Epidemiology and control of emerging strains of poultry respiratory disease agents" examines poultry disease through diagnosis as well as immune and prophylactic strategies rather than genetic aspects of resistance and immunity. Similarly, the NC-187 project, "Enteric diseases of poultry" explores the specific area of enteric disease but without an emphasis on genetics. The S-233 project "Genetic relationships to growth and reproduction in diverse poultry populations" focuses on genetic variation in performance traits rather than disease and immunity in poultry. The NC-168 project "Advanced technologies for the genetic improvement of poultry" may be the most related to NE-60. The major histocompatibility complex (MHC) genes and the endogenous viral (*ev*) genes will be studied in both projects. Although these two gene families are being examined, the two projects are quite distinct in their objectives and application of work with these genes. The NC-168 project focuses on identification and mapping of poultry genes as well as strategies for incorporating new genes into selection programs. By contrast, the NE-60 project studies MHC and *ev* genes to elucidate of their role in genetic resistance and immunity in poultry. The NRSP-8 National Animal Genome Research Program emphasizes mapping the genomes of various agriculturally important animal species including chickens.

Several stations (CA, IA, ARS-ADOL, NC) have representatives on both NC-168 and NE-60 technical committees. This representation will enhance communication to coordinate complementary efforts, encourage appropriate joint efforts and avoid duplication. For example, lines selected for particular characteristics by NE-60 may be used for NC-168 genome mapping efforts, whereas transgenic chickens or new selection methods developed by NC-168 may be examined for use in immune response and disease resistance studies in NE-60. To further increase coordination of efforts and timely sharing of information, NE-60 and NC-168 technical committees have held joint meetings in the past.

Two other U.S. laboratories outside of NE-60 conduct research relevant to genetics of immunity and disease resistance in poultry. Both of these stations are NC-168 members. Ohio

has studied the genetics of disease resistance in turkeys and the turkey MHC. Virginia has selected lines for antibody response to SRBC and has used these lines to examine the relationships among blood groups, MHC and disease resistance.

A historical knowledge base has been readily available that the disease resistance of commercial chickens can be improved through genetic selection. In terms of improvement of a variety of economic traits, including genetic resistance to disease, commercial poultry breeders lead breeders of other species. Recent scientific discoveries including applications of recombinant DNA and hybridoma technology, progress in understanding the immune system, and enhanced knowledge about poultry pathogens, promise imminent, significant improvements in poultry health and production efficiency.

Several laboratories have made progress in elucidating the molecular structure of MHC genes and antigens. Continued work in this area is needed to define additional polymorphisms, regulation of gene expression and relationships with resistance to disease. In addition, the new region, designated *Rfp-Y* containing MHC-like genes, was discovered in this project period. Augmented identification of beneficial alleles for resistance to Marek's disease virus, Rous sarcoma virus, *Salmonella enteriditis*, and *Eimeria* spp. has resulted from studies of the MHC association with many diseases. The repertoire of pathogens under study must be expanded because these diseases represent only a small fraction of the pathogens that can affect poultry. A more detailed understanding of the pathogen-host relationship is also needed. Most studies have utilized genetic stocks of the egg-laying type but this work is now expanding into the meat-type birds. All these facets of chicken MHC research will be addressed by the proposed studies in this project renewal.

The NE-60 regional research project scientists design, create, maintain, and study unique genetic lines of poultry. Some members carry out all of these functions and others a subset as an integral part of our research. These efforts have been our contribution as well as our responsibility to carry out the objectives of the project to understand the genetic basis for immunity to disease effectively and efficiently. Our tradition has been to share genetic resources. The extant genetic lines available for collaborative research are at risk at many research stations. Loss of avian genetic resources established over the last 70 years will impact our community of collaborators as well as the avian research community at large. It is unlikely that once lost, those unique resources now in hand (e.g., congenic, recombinant, and inbred lines) will be recreated. Therefore, it is imperative to conserve the resources currently available. The technical members of the project recognize the import of this issue and several of our Technical Committee Members serve on the Avian Genetic Resources Task Force. The members are committed to the establishment of a national system of networked researchers and a site for conservation of orphaned stocks to support our objectives of understanding and improving resistance to disease in poultry.

Innovative technologies have been effectively used to identify and characterize many cell-surface antigens related to immune function. These methods include Mabs and DNA probes. These techniques expand upon the pioneering chicken blood-group work conducted by NE-60 members decades ago, which continues to be an important and readily applicable technology today in both research and industry. Further research on non-challenge methods to select for disease resistance in poultry must, and will, continue in the NE-60 project. All of these facets of NE-60 research will increase animal well-being, improve food safety and aid in resource conservation by enhancing production efficiency through genetic selection.

OBJECTIVES

1. Identify and characterize genes and their relationships to disease resistance in poultry with an emphasis on the major histocompatibility complex as well as other genes encoding alloantigens, communication molecules and their receptors and other candidate systems.
2. Identify and characterize environmental, dietary and physiologic factors that modulate immune system development, optimal immune function and disease resistance in poultry genetic stocks.
3. Develop and evaluate methodologies and reagents to assess immune function and disease resistance to enhance production efficiency through genetic selection in poultry.

PROCEDURES

OBJECTIVE 1: Identify and characterize genes and their relationships to disease resistance in poultry with an emphasis on the major histocompatibility complex as well as other genes encoding alloantigens, communication molecules and their receptors and other candidate systems.

Evaluation of resistance and/or susceptibility to disease in chickens has been facilitated by the development of chicken strains genetically-selected for the study of various gene systems. One such gene system in chickens is the major histocompatibility complex (MHC) or *B*-complex, a group of genes whose products regulate the immune response. Special chicken strains have been produced by selection for certain *B* haplotypes during backcrossing to an inbred line. These strains which differ only at the *B*-complex are called B-congenic lines (**CA, NH, IA, USDA-ARS-ADOL**).

In addition to the *B*-congenic lines, highly inbred lines and experimental chicken lines selected for unique immune functions are available for research purposes (**AL, AR, CA, NH, NIU, NY-C, IA, USDA-ARS-ADOL**) [Appendix 1]. These populations have served as valuable genetic resources for research conducted by collaborating institutions in NE-60, as well as by other research laboratories. Because of the existence of specific facilities, expertise and genetic resources at individual stations, many studies are appropriately conducted at one location. Yet such studies may involve shared resources or expertise. The ability to discern which of the resulting discoveries are generalizable comes from the planning, coordination, cooperation and sharing of data, which takes place through participation in this regional project.

The **NH** station will continue the production and maintenance of the 6.*B* congenic chickens. These congenic lines, 6.6-*2* (B^2B^2) and 6.15-*5* (B^5B^5) on the **USDA-ARS-ADOL** inbred line 6_1 background genome are being maintained separately after completing ten backcrosses. In a collaborative effort with **NIU**, **NH** will use line 6.15-*5* to identify background gene effects that may influence src DNA and Rous sarcoma tumor growth and metastasis. Line 6.15-5 (B^5B^5) chickens have progressive src DNA tumor growth and metastasis compared to line TK ($B^{13}B^{21}$) chickens that have regressive tumor growth and rare metastasis. A cross of the two lines followed by a backcross to line TK will produce B^5B^{21} and B^5B^{15} chickens. These birds will be mated with *inter se* to yield experimental chickens, which segregate for the MHC and have 75% of the TK background. Tumor growth and metastasis from either src DNA or RSV *src* DNA tumors will be examined. Of particular interest will be B^5B^5 chickens which will be compared for their response to line 6.15-*5* chickens, the line of origin for B^5B^5.

NH will test RSV and v-*src* tumor growth in birds having different background genes in the context of $B^{17}B^{17}$ MHC haplotype. Chickens of many different types will be crossed to line UCD 003 ($B^{17}B^{17}$). All progeny will be MHC heterozygotes. The F_1 progeny will be backcrossed to line UCD 003 ($B^{17}B^{17}$) to produce $B^{17}B^{17}$ birds that contain 75% of the line UCD 003 genome. These birds will be randomly mated to produce experimental chicks that will be injected with either *src* DNA or Rous sarcoma virus. Tumor growth, immunity and metastasis will be assessed for effects of background genes. NH will also continue experiments on *src* tumor growth, immunity and metastasis using Rous sarcoma virus, src DNA constructs and chimeric DNA constructs containing components of v-*src* and c-*src*.

Eight *B*-congenic lines were developed in inbred line $15I_5$ and they are maintained at the USDA-ARS-ADOL. These lines will be used to produce additional antisera to B-FIV (MHC) antigens common in White Leghorns, as well as to those common to broilers using procedures developed in 1996. The use of the *B*-FIV antisera in commercial strains will be evaluated. The effects of the *B*-haplotype on vaccinal immunity to vaccines other than MD will be established in collaborative studies using the *B*-congenic chickens. In addition, USDA-ARS-ADOL has developed nineteen recombinant congenic strains (RCS) of chickens by backcrossing inbred line 7_2 chickens to inbred line 6_3 chickens. After two backcross generations, full-sib matings were conducted in each of 19 families; it is estimated that each 6C.7 RCS will contain a different 12.5% of the line 7_2 genome. These 19 RCS of chickens will continue to be developed by inbreeding. Since line 7_2 is susceptible to MD and lymphoid leukosis tumors and line 6_3 is resistant to tumor development, and these lines have the same MHC (B^2) haplotype, it is hoped that the resulting RCS strains will help identify non-MHC genes influencing tumor resistance, as well as to define other genes influencing primary lymphoid organ development, e.g. cytokines.

NIU has previously identified and maintained chickens that are recombinant in the MHC and these MHC genotypes are being placed in the line UCD 003 ($B^{17}B^{17}$) background genome to develop the 003.R congenic recombinant chickens by NH. Females possessing each *B* recombinant type (R1-R6) mated to UCD 003 males will be in the tenth backcross generation in 1997. Two other recombinants, received from Hans Abplanalp (CA), are also maintained by NH. Line 386 is $B^{F15\text{-}G21}B^{F15\text{-}G21}$, whereas Line 387 is $B^{F21\text{-}G15}B^{F21\text{-}G15}$.

The NH station is developing *Rfp-Y* (MHC-like) congenic chickens. Inbred line UCD 003 ($B^{17}B^{17}$) and Line UCD 001 ($B^{Q}B^{Q}$) were mated, followed by two backcrosses to line 003. Some progeny were heterozygous for both the *B* (B^{17} and B^{Q}) and *Rfp-Y* systems. These birds will continue to be backcrossed to line UCD 003. The objective is to produce congenic lines which have two *Rfp-Y* types expressed with two different MHC types on the background of line UCD 003. In a collaborative project involving NH and M. Miller (Beckman Research Institute), RSV and v-*src* tumor growth will be examined in *Rfp-Y* congenic birds. Birds with either $B^{17}B^{17}$ or $B^{Q}B^{Q}$ MHC haplotypes, which are heterozygous for Rfp-Y, will be crossed to produce segregation for *Rfp-Y*. These progeny will then be injected with either *src* DNA or Rous sarcoma virus. Effects of *Rfp-Y* on tumor growth, immunity and metastasis will be assessed.

Seven polymorphic alloantigen systems (*A, E, C, H, I, L* and *P*) will be tested for their interaction with the MHC and *Rfp-Y* systems. NIU has established specially-designed matings between the Ancona stock and the White Leghorn stock. Line-cross chicks from these matings will then be evaluated in collaborative studies for Marek's disease (CA), Rous sarcoma (NH) and coccidiosis (NH). Complete characterization of alloantigen systems, other than MHC and *Rfp-Y* will also involve protein purification and gene cloning (NIU and M. Miller, Beckman

Research Institute). Polymorphic genetic systems in pheasants, bobwhite quail and other captive species will also be evaluated by **NIU**.

IA will continue to develop, maintain and monitor their genetic populations of chickens. There are 14 different highly inbred lines, many in MHC-congenic pairs, and many of defined disease-resistance characteristics. There are four sublines of the S1 partially inbred line, differing in the MHC region (B^1 or B^{19} by serological typing, and Ir-GAThigh or Ir-GATlow by antibody measurement). The **IA** station has also backcrossed the four different S1 MHC haplotypes onto the G-line background, resulting in a series of six MHC-congenic lines (including the two original B types, B^6 and B^{13}) on the G inbred line background. The **IA** lines will be used to characterize MHC and non-MHC genetic effects on immune responses, such as kinetics of antibody production to a variety of antigens and on resistance to Marek's disease induced by highly oncogenic strains of MD virus.

The **AL** station will continue to characterize broiler *B* haplotypes at the molecular level and they will extend their analysis to additional lines. Broilers differing in *B* haplotype will be compared for susceptibility to various economically-important diseases, including infectious bursal disease (IBD), cecal coccidiosis and necrotic enteritis. The work with IBD will be conducted in collaboration with Dr. F. J. Hoerr (**AL**) and the cecal coccidiosis project will include Dr. R. A. Norton (**AL**).

The **DE** station will characterize MHC haplotypes at the molecular level in a commercial broiler population. In a collaborative project with **NIU**, **DE** will produce alloantisera in broilers for identification of specific broiler MHC haplotypes. These antisera will be tested against a panel of reference blood cells maintained in chicken populations at **NIU** to determine the evolutionary relationships between egg laying and meat-type chickens. **DE** will evaluate the influence of broiler MHC haplotypes on innate and vaccinal immunity to Marek's disease. In addition, **DE** will evaluate the role of non-MHC genes in resistance/susceptibility to disease Marek's and coccidiosis (in collaboration with **USDA-ARS-LPSI**).

NH will continue production of the B^{19} trisomic chickens. Trisomic progeny, $B^2B^{15}B^{19}$ have been mated to $B^{19}B^{19}$ chickens to yield both $B^2B^{19}B^{19}$ and $B^{15}B^{19}B^{19}$ trisomics. These two trisomic types will be crossed to $B^{19}B^{19}$ chickens to produce $B^{19}B^{19}B^{19}$ chickens. The trisomic chickens will be useful in studies of gene dosage effects of the B^{19} haplotype. In a collaborative study (**NH** and **NY-C**), the effect of the B^{19} gene dose on response to Marek's disease will be examined. Trisomic $B^{19}B^{19}B^{19}$ chickens will be crossed to $B^{19}B^{21}$ chickens to produce MHC haplotype and gene dose segregation. Progeny will consist of four types ($B^{19}B^{19}$, $B^{19}B^{21}$, $B^{19}B^{19}B^{19}$ and $B^{19}B^{19}B^{21}$). These individuals will be injected with Marek's disease virus after which the severity of the disease will be determined.

The **FRAMINGHAM** station has identified an influence of the chicken *B*-complex on immunity to *Salmonella enteritidis*. Since MHC resistance to S. enteritidis is expressed early, it is unlikely due to the production of specific antibody. **FRAMINGHAM** will determine the role of complement in *S. enteritidis* resistance by determining if differences in its activity can be detected among B-congenic lines already identified as susceptible, intermediate and resistant.

The genetic basis for the expression and activity of nitric oxide synthase (NOS) in chicken macrophages will be assessed by the **NC** station. Three genetic lines, Cornell K-strain ($B^{15}B^{15}$), GB1 ($B^{13}B^{13}$) and GB2 (B^6B^6) will be used to compare inducible and constitutive levels of NOS expressed in macrophages. Preliminary studies have categorized these three lines as high and low responders for iNOS activity. By utilizing disease challenges such as coccidia or

Rous sarcoma (collaboration with NH), correlation of iNOS expression with disease pathogenesis will be established.

NH will assess the influence of endogenous viral gene 3 on oncogene tumor growth. Line EV-3 possesses endogenous viral gene 3 (*ev*-3, $B^{21}B^{21}$), whereas Line EV-0 ($B^{21}B^{21}$). possesses no endogenous viral genes. Rous sarcoma virus or *src* DNA tumor growth in both lines will be compared for (ev-3) effects. **USDA-ARS-ADOL** will develop new assays to quantitate the amount of EV present in chicken plasma and to predict the genotype of chickens resistant to EV using an alloantiserum that detects new derivatives. The influence of EV in broilers on susceptibility to subgroup J avian leukosis viruses that cause myeloid tumors will be assessed using the R2 antisera and an assay that detects EV in plasma.

To establish the effects of an individual gene from a chicken, or from a pathogen, on disease resistance, the development of transgenic chicken technology is needed. Chicken MHC genes and MD viral genes are under development for transgenic insertion into the chicken genome. Collaborative studies are underway with **USDA-ARS-ADOL** and Dr. R. Etches (Guelph) to establish the transgenic methodology that will allow insertion of the developed genes into the chicken genome. If a transgenic chicken is developed expressing an introduced gene, it will be reproduced to study the effect of the inserted gene on the disease of interest, e.g. MD.

The **CA** (Delany) station will characterize the ribosomal (r)DNA genotypes of genetically-defined chicken populations with particular MHC haplotypes. The objective of this research is to establish if rDNA genotype affects disease resistance and susceptibility. Initial research will focus on two disease model systems: sarcoma induction by v-src and lymphoma induction by MDV. **CA** (Delany) has shown that the rDNA (which is linked to the MHC) consists of distinct "families" of the tandemly repeated rRNA gene repeat units and further, that different genetic lines exhibit different rDNA genotypes. **CA** (Delany) will continue collaborative research with **NH** where 6.*B* congenic chickens will be injected with a v-src construct. Baseline rDNA genotypes of the congenic B^2 (regressor) and B^5 (progressor) birds will be established in terms of the number of gene repeats as well as the type and distribution of repeat units. Baseline rDNA genotypes will be compared to primary wing web and secondary tumors (sarcomas) to establish if changes in rDNA genotype occur concomitant with tumor progression. Nucleoli (number and morphology) from normal tissue, primary and secondary tumors will be examined to establish if, as in the case of numerous human tumor systems, nucleolar morphology is pathologically disrupted. In addition, **CA** (Delany) will begin to characterize rDNA genotypes of genetically defined populations known to be susceptible or resistant to MDV in collaboration with **CA** (P.Wakenell). The baseline rDNA genotypes associated with MDV-resistant and susceptible MHC haplotypes will be characterized. The baseline rDNA genotypes and nucleolar phenotypes will be compared to the rDNA genotypes and nucleolar phenotypes of MDV-induced lymphomas.

The **CA** station (Delany) will conduct research to establish the linear orientation of the MHC, rDNA, *Rfp-Y* complexes and their location in relation to the centromere and telomere regions on microchromosome number 16. The MHC, *Rfp-Y* and rDNA have been shown to be linked by cytogenetic trisomy mapping, cosmid clone analysis, and metaphase chromosome physical mapping; however, these complexes show independent segregation in inheritance studies. The rDNA has been suggested to be a "hotspot" for recombination and to be located between the MHC and *Rfp-Y*. A new mapping mode (which offers enhanced resolution as compared to metaphase chromosome analysis) exists in the form of fluorescent in situ hybridization of extended chromatin fragments (ECFs). ECFs prepared from germline and

somatic cells will be hybridized with, rDNA, MHC, *Rfp-Y* (M. Miller, Beckman Research Institute) and telomere probes to establish by physical mapping the orientation of these regions in relationship to one another.

Research at **GA** will establish the importance of natural killer (NK) cell-like activity in the innate ability of chickens to resist Marek's disease virus (MDV) infection. Further work will then determine whether modulation of this natural immunity (NK cell activity) could be used as part of Marek's disease prevention programs, thereby providing a means to reducing industry losses. Cytotoxicity assays for NK-like activity of chickens having MHC haplotypes reported to be MDV resistance, N-2a chickens (B^{13}), and MDV sensitive, P-2a chickens (B^{21}), indicate N-2a chickens have greater killing capabilities by NK cells than the P-2a chickens (**GA**). The N-2a chickens displayed greater killing at one, two, and three weeks of age, with the increase becoming significant by the third week. **GA** has also compared NK activities in two commercial lines of broilers: Arbor Acres and Perdue. Between these two broiler lines we found the Perdue birds to have significantly higher natural cytotoxic ability.

GA will correlate MHC-restricted cytotoxicity with NK cell function and the ability to resist MDV infection during the early cytolytic and immunosuppression phases. Pinpointing the particular cellular mechanism will explain why NK activity correlates with resistance, and therefore allow targeting for immune modulation or vaccination production. **GA** will determine the degree of immunosuppression caused by MDV by assessing MHC-restricted cytotoxicity. Low MHC-restricted cytotoxicity with high NK activity in genetic lines of chickens established as resistant to Marek's disease is expected whereas high NK activity during the early immunosuppressive phase will determine recovery from infection. This will reinforce the role of NK cells during the early phase of MDV infection. The *in vitro* findings will be confirmed with a vaccination/challenge experiment.

OBJECTIVE 2: Identify and characterize environmental, dietary and physiologic factors that modulate immune system development, optimal immune function and disease resistance in poultry genetic stocks.

Immunomodulation during microbial and autoimmune insult would remain the focus of several stations. **AR** will continue studies on autoimmune vitiligo in Smyth line (SL) chickens. Special emphasis will be placed on identifying environmental factors required for the expression of vitiligo in genetically susceptible SL chickens and on immune mechanisms involved in autoimmune destruction of pigment cells in SL vitiligo. Immune profile of turkeys suffering from an enteritis and mortality disorder, PEMS, as a result of multifactorial microbial and environmental exposure would be determined (**NC**). Lymphoid, myeloid, and cytokine profiles will be correlated with PEMS (**NC**). The influence of endogenous viral gene 3 on chicken macrophage function will be assessed (**NC, NH**). Line EV-3 possesses endogenous viral gene 3 (*ev*-3) which is inserted in the chicken *hck* gene. Transcripts of hck are increased in *ev*-3+ birds. Line EV-0 has no endogenous viral genes. Both of these lines are $B^{21}B^{21}$. Because of high *hck* expression in macrophages, these lines will be compared for macrophage functions to determine if *ev*-3 improves cellular function.

NY-C will pursue studies of thyroid/thymus interactions. Supplementation with a low level of T3 increased IL-2 activity and IL-2 receptor expression whereas higher T3 levels downregulated the cytokine and its receptor. Thymulin supplementation had a similar effect. Experiments will focus on modulation of thyroid hormone and/or thymulin to determine their effects on functional immune responses. Higher thymulin decreased autoantibody production

and lymphoid infiltration in autoimmune OS strain chickens. These studies will be extended to other systems. Cytokines and their receptors, such as IL-2, will be measured following hormonal manipulation.

Three stations will examine modulation of avian immune system by dietary factors. The University of Arkansas (**AR**) will examine the extent and mechanisms by which vitamin E and other antioxidants affect immune system development and function in young commercial broiler and turkey poults. Previously reported findings that vitamin E enhances several baseline immunological endpoints after *in ovo* exposure (Gore and Qureshi, 1997) would now be extended by imposing a disease challenge on turkeys while on various dietary or *in ovo* -exposed vitamin E levels (**NC**). In addition, possible immunomodulatory effect of dietary vanadium would be examined in greater details to determine possible mechanism(s) at the cellular and molecular level (**NC**). Experiments are planned to continue the investigation of dietary amino acids and their effects on the immune system in the infectious bronchitis virus model (**NY-C**).

Significant progress is being made to determine the molecular and functional nature of immunologicallly-relevant avian cytokines and metabolites. **NY-C** has shown that the maintenance of latency of MDV is, at least in part, influenced by cytokines such as the latency-maintaining factor (LMF) (Buscaglia and Calnek, 1988) and interferons (Volpini *et al.*, 1995, 1996, Kaplan *et al.*, 1997). Increased MDV replication can lead to immunosuppression and transformation of T cells. **NY-C** would continue to examine the role of cytokines in Marek's disease by looking at the mechanism(s) involved in the cytokine-induced maintenance of latency by transfecting cells with plasmid constructs in which promoter/enhancer regions of different MDV genes are replaced in front of the luciferase gene. Modulation of luciferase expression by the addition of recombinant chicken interferons will be examined. In addition, the effect of interferons on specific MDV transcripts in MD cell line MDCC-CU184 will be quantitated. Relationship of cytokine profile(s) with the outcome of poult enteritis and mortality syndrome will be examined (**NC**). Alterations in the immune profiles would be endpoints to examine the possible involvement of immunosuppressive microbial agent(s) such as viruses (**NC** and **NY-C**).

Studies would continue to determine the genetic-immune interaction using chicken lines that were divergently selected for high and low antibody against SRBC (**PA**). The relationship between MHC as well as level of circulating SRBC antibody and different immune responses will be studied in these lines. Mechanisms involved in the initiation of cell-mediated immune responses will be studied in Cornell-K-strain chickens. The effect of *in vitro* and *in vivo* melatonin on different immune responses will be studied under different photoperiod regimens in broiler chicks. Some of the immune parameters that will be measured will include production of lymphokines and heat-shock proteins. Furthermore, broiler and laying chicks at different ages will be examined for the production and function of heat-shock proteins under different stressful conditions such as temperature, atmospheric ammonia and ACTH.

Since it has been established that dietary immunomodulation is possible in the PHA system, it would be of interest to determine if this phenomenon extends to systems employing pathogenic challenge. Additionally, it may be important to determine if other dietary additive substances might be of use as immunopotentiators (**FRAMINGHAM**).

GA will determine whether modulation of the bird's natural killer cell numbers and activity, through naturally-occurring food/water additives, could be utilized as an inherent trait in Marek's disease prevention. The efficacy of five possible immune modulators will be studied. Because doses are extrapolated from studies using these modulators in animals, all the modulators will be titrated in the Arbor Acres broiler line. Once an immunomodulator has been

established to increase NK-like activity a vaccination and challenge-type trial will be done. The RB1B virus ID^{50} in both broiler and leghorn chickens will be titered prior to the final challenge experiment. This will allow "fine-tuning" of disease production and detection of differences in our experimental groups.

Many studies may be conducted at individual stations to take advantage of specific facilities, expertise and genetic resources. Shared resources or expertise come from other regional project members through effective planning, cooperation and sharing of data which are essential elements of the project.

OBJECTIVE 3. Develop and evaluate methodologies and reagents to assess immune function and disease resistance to enhance production efficiency through genetic selection in poultry.

Genetic stocks will be analyzed to determine the genetic correlation between antibody levels and resistance to disease (IA). Birds will be evaluated for their antibody production as well as their capacity to resist disease. The genetic correlations between these characteristics will be calculated.

The IA station will identify "anonymous" DNA markers (e.g., microsatellites) associated with antibody production and disease resistance. Various genetic stocks will be evaluated through standard procedures. Selected stocks will be evaluated for specific differences in microsatellites markers. In addition, F2 populations may be evaluated for segregation of markers associated with the level of antibody production and disease resistance.

Alloantisera for chicken cell-surface antigens will be produced and characterized (IA). Antisera are already produced to type MHC antigens in various stocks. Other cell surface antigens that may influence immunity will be identified with alloantisera produced by injecting recipient chickens with cells for donor chickens. These unique cell-surface antigens may directly or indirectly affect disease resistance.

The utility of lectin containing bean extracts in the study of the humoral immune response development will be extended. Application of these extracts to the characterization and differentiation of protective antibody from those types that might actually participate in the disease process itself will be given special emphasis (FRAMINGHAM). Individual stations utilize their specialized facilities, genetic resources and expertise while relying on shared assets from other regional project members to increase research effectiveness and productivity.

The finding that genetically resistant N2a chickens are able to generate cytotoxic T lymphocytes (CTL) raises the question if other resistant lines are also able to generate ICP4-specific CTL while other susceptible lines are unable to do so. NY-C plans to test this hypothesis by generating reticuloendotheliosis (REV) cell lines from chickens with different MHC class I haplotypes, which have been or can be characterized for resistance to MD. Some of these lines have already been obtained or will be obtained from NE-60 stations or collaborators (IA, NH, USDA-ADOL, and NIU). In addition, NY-C has obtained eggs from AG-Canada and have established two flocks which carry the $B^{21}B^{21}$ or $B^{19}B^{19}$ haplotype. A number of REV-transformed cell lines have been established from the different genetic chicken lines. The plan is to stably transfect these lines with ICP4 or glycoprotein B (gB) from MDV. The latter will serve as a positive control because gB-expressing cells are lysed by CTL from the P2a and N2a lines. These cell lines will be used to test the hypothesis that ICP4 is recognized by CTL from genetically resistant but not susceptible chicken lines.

NY-C also plans to develop a system to stimulate antigen-specific CTL in vitro using primary CTL from spleen cells in order to use enriched populations of these cells for passive transfer studies. Bone marrow cells will be transformed with a plasmid containing rel fused to an estrogen receptor. The basic construct has been provided by Dr. Zenke (Berlin, Germany). Transformed bone marrow cells will be stably transfected with the MDV gene(s) of interest. Manipulation of the culture media will result in the development of dendritic cells. These cells will be used as antigen-presenting cells for *in vitro* stimulation of primary CTL.

For passive transfer experiments, NY-C plans to generate primary CTL using recombinant fowl poxvirus expressing the relevant genes of interest followed by *in vitro* restimulation and expansion. Chickens will be challenged with MDV followed by inoculation of MHC-matched CTL. The CTL effectiveness in curtailing virus replication will be measured using a quantitative PCR assay. These enriched populations of antigen-specific CTL will be used to determine the nonapeptides recognized by CTL from MDV-vaccinated chickens. Briefly, truncated constructs will be made for the genes coding for proteins recognized by CTL. These genes will be cloned into eukaryotic expression vectors and transfected into REV lines which will serve as target cells using either primary CTL or CTL after *in vitro* enrichment as effector cells. After identifying the gene fragment of interest, a series of overlapping nonapeptides will be obtained from our collaborators at the ID-DLO in the Netherlands. These nonapeptides will be mixed with REV cell lines and used as target cells to determine which nonapeptdies are recognized by CTL. The use of *in vitro* stimulated secondary CTL will greatly facilitate these studies. The ultimate goal is to determine which nonapeptides are important for vaccine-induced resistance and to determine if there are differences in nonapeptide recognition between chicken strains based on MHC differences.

EXPECTED OUTCOMES

Project members will continue to maintain unique genetic lines that are invaluable to the project itself and to the research community at large. Additional genetic material will be developed as needs arise. New information is anticipated about resistance to Marek's disease, coccidiosis, oncogene tumors as well as other diseases. The major histocompatibility complex, ribosomal genes and other systems will be further characterized by molecular methods. Other advances are expected in understanding autoimmune diseases, immune system/hormone interactions, cytokines and modulation of immunity by dietary factors. During the tenure of the project, it is expected that stations will continue direct biotechnology applications to identify factors affecting disease resistance and immunity. New methods including the use of transfected cell lines expressing antigenic components of disease agents and identifying genes associated with antibody production or disease resistance via microsatellite DNA markers will be enhanced. The combined efforts of the NE-60 stations will generate new information communicated through publications, proceedings and presentations. These endeavors will improve poultry production through increased immunity and disease resistance.

ORGANIZATION

The planning and supervision of the NE-60 Regional Research project shall be the responsibility of the Regional Technical Committee. The membership of this committee shall consist of an Administrative Advisor, a technical representative of each participating agency or experiment station, and a representative of the USDA Cooperative State Research Education and Extension Service (CSREES). The voting membership shall consist of the Technical Representatives; only one member of each participating agency or experiment station shall be eligible to vote.

The Technical Committee shall be responsible for review and acceptance of contributing projects, preparation of reviews, modification of the regional project proposal, and preparation of an annual report for transmittal by the Administrative Advisor upon approval to CSREES. Annual written reports will be prepared by each technical committee member and distributed at the annual meeting. A limited number of the compiled annual reports will be available upon request from the Administrative Advisor.

The Technical Committee will meet yearly and elect a secretary, who will serve the year after election and as the chairperson the following year. An Executive Committee will be formed to conduct all business of the Technical Committee between annual meetings. The Executive Committee shall consist of the current Technical Committee Chairperson, the Secretary, and the two immediate Past Chairpersons.

The chairperson may name other subcommittees as needed to perform specific assignments. They may include subcommittees to develop procedures, manuals, and phases of the regional project, to review work assignments; to develop research methods, to prepare publications, and to write proposals.

Other agencies and institutions may participate and vote at the invitation of the Administrative Advisor. Minimum expectations for Technical Committee members is attendance at an annual meeting at least one year out of two, and submission of a written annual report every year. Collaborators may include emeritus members with an interest in attending annual meetings, scientists who wish to contribute and participate by virtue of having a special skill or interest, and those who participate in research with a special focus or interaction with an individual Technical Committee member.

TITLE: Genetic Bases for Resistance and Immunity to Avian Diseases

Administrative Advisor - Kirklyn M. Kerr — Date: 4/7/98

Chair, Regional Association of Directors — Date: 4/7/98

Administrator,
Cooperative State Research, Education, and Extension Service — Date: 6/25/98

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Resources:

Agency/Institution	SY	PY	TY
*AL	0.40	0.50	0.50
*AR	0.20	0.50	0.50
*CA	0.10	0.70	0.25
*CA	0.35	0.70	0.50
*DE	0.25	0.30	0.00
*GA	0.10	0.00	0.15
*IA	0.20	0.50	0.70
FRAMINGHAM	0.25	0.00	0.00
ARS-BARC	0.20	0.20	0.50
ARS-ADOL	1.00	1.00	1.00
*NH	0.70	0.50	0.50
*NY-C	0.10	0.50	0.25
*NY-C	0.20	0.80	0.50
*NY-C	0.25	0.00	0.20
*NC	0.10	0.05	0.10
*PA	0.25	0.00	0.35
TOTAL	4.65	6.25	6.00

*State Agriculture Experiment Stations