Genetics, Immunoresponsiveness, and Disease Resistance in Chickens

by

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INTRODUCTION

Economic losses to the poultry industry due to mortality and reduced productivity from infectious agents constitute 10 to 15% of the annual costs to poultry producers (Gavora and Spencer, 1983). These losses, while substantial in themselves, may be trivial compared to the possible longterm losses in genetic potential, as seen in the case of lymphoid leukosis (Spencer et al., 1979; Gavora et al., 1980).

Disease prevention programs include vaccination regimes, medication in the feed, superior husbandry, and breeding for disease resistance. Vaccination, however, may lead to reduced genetic potential for disease resistance (Hutt, 1958), and continuous use of feed additives may alter host-pathogen interactions, leading to resistance by pathogens, which may have implications for human health (Holmberg et al., 1984). Superior husbandry such as the pathogen-free environments used by primary breeders reduces exposure of the birds to infectious agents and creates an atmosphere in which natural selection for disease resistance is reduced. Artificial selection for disease resistance should be conducted so as not to reduce the genetic potential for production traits (Gavora and Spencer, 1983).

Historically, efforts to select for disease resistance in animals concentrated primarily on resistance to specific diseases (see reviews by Gavora and Spencer, 1978; van der Zijpp, 1983a; Hartmann, 1985) or on livability (Gavora and Spencer, 1978). Recent expansion of knowledge in

this area has resulted from research involving selection for response to complex antigens such as sheep erythrocytes (Siegel and Gross, 1980; van der Zijpp and Nieuwland, 1986); increased lymphoid tissue mass (Yamamoto and Glick, 1982); marker genes known to be associated with disease resistance, especially the major histocompatibility complex (Kim et al., 1987); response to multiple antigens of different types (Pitcovski et al., 1987); and other immunological parameters (Okada and Yamamoto, 1987).

Advances in gene transfer technology have implications for poultry breeding. Current breeding procedures show progress in economically important traits of 2 to 3 percent per generation and gene transfer improvements require a 5 to 10 percent improvement to be competitive (Smith, 1988). Perhaps more important, however, is whether the improvements from such procedures are consistent across populations. In chickens, the sex-linked late feathering and dwarfing alleles *K* and *dw* have shown potential in meat but not egg stocks (e.g. Merat, 1982; Harris et al., 1984). In the case of the former, there is an association with lymphoid leukosis while for the latter reproductive rate is reduced. Underlying biological reasons for these results are generally unknown and warrant investigation before implementation of gene transfer techniques in poultry breeding programs.

This dissertation consists of three phases interrelating immune response with characters of commercial importance in chickens. They involve comparisons of lines selected for high and low antibody production to sheep erythrocytes for a) factors affecting immunoresponsiveness and disease resistance, and b) correlated responses of production traits to

selection. Lines selected for high and low juvenile body weight and sublines to which the *dw* allele had been introduced were compared for antibody responses to sheep erythrocytes and physiological parameters which might affect such responses.

A list of abbreviations used in this dissertation is presented on page 128.

REVIEW OF LITERATURE

A quantitative trait may be affected by minor and major genes, adding incrementally to influence its overall phenotypic expression. Immunoresponse measures have been demonstrated through selection experiments to be quantitatively inherited, with major genes also playing an important role. This review will address both major loci and general effects as they pertain to the topic of this dissertation.

Major loci

Major histocompatibility complex (MHC). During this decade, considerable research with chickens has focused on the effects of erythrocyte alloantigen systems on disease resistance (e.g. Briles et al., 1980; Johnson and Edgar, 1984; 1986; Bacon, 1987). Most studies have involved the *B* locus, actually a complex of very tightly linked genes with three major regions referred to as the *B-L*, *B-G*, and *B-F* regions (Pink et al., 1977). The *B* system, which is the MHC of chickens (Schiermann and Nordskog, 1961), is located on a microchromosome and is linked to a nucleolar organizer region (Bloom and Bacon, 1985). Region *B-L* codes for Class I MHC antigens, *B-F* for Class II antigens, and *B-G* for antigens on erythrocytes, which are denoted Class IV antigens and are unique to avian species (Vainio et al., 1987). Histocompatibility effects of the *B* system are associated with the *B-F* region.

Crossovers within the *B* system, while rare, do occur (e.g. Schiermann and McBryde, 1969). Because such crossovers usually occur between the

B-G and *B-F* regions, and *B-G* antigens on erythrocytes are commonly used markers for MHC haplotypes in the chicken, some haplotypes have been identified as having the same *B-F* and/or *B-L* regions. B^4 and B^{13} share both *B-F* and *B-L* regions (Briles and Briles, 1987). Data on the *B-L* region are limited, but there may be identity in that region of B^{12} and B^{19}. The *B-G* region of B^2 and B^{12} is identical, and that of B^3 and B^{14} may be identical (Briles and Briles, 1987).

The most widely studied effect of the MHC is its association with resistance to neoplastic disorders, including Marek's disease (Briles et al., 1980), lymphoid leukosis (Bacon et al., 1981), and Rous sarcoma tumors (Collins et al., 1977; Schiermann et al., 1977). Resistance to Marek's disease is associated with the *B-F* region of the MHC (Briles et al., 1983). Chickens of haplotypes B^2, B^6, and B^{21} showed moderate to strong resistance to challenge with Marek's disease virus, while those with haplotypes B^3, B^5, B^{13}, B^{15}, B^{19}, and B^{27} were highly susceptible to such a challenge (Briles et al., 1980). Heterozygotes with allele B^{21} were more resistant to Marek's than other heterozygous combinations. The B^2B^{21} heterozygotes were especially resistant, surpassing the other B^{21} heterozygotes, and approaching the B^{21} homozygotes in resistance (Briles et al., 1982a). In other crosses of Leghorn lines, resistance to Marek's disease exposure by offspring of B^2, B^{13}, B^{14}, or B^{21} parents depended upon the line to which they had been crossed. Offspring did not differ in mortality when those parents were crossed with a line homozygous for B^{15}, but when crossed with a line homozygous for B^2 the offspring of B^{21}

parents had lower mortality than those of B^2, B^{13}, or B^{14} parents (Hartmann et al., 1986).

Chickens homozygous for B^2 regressed Rous sarcoma tumors more quickly than did B^5 haplotype chickens (Collins et al., 1977). Concurrent studies (Schiermann et al., 1977) showed that the B^2B^2 haplotype regressed Rous sarcoma tumors while the B^1B^1 haplotype did not. Backcross experiments showed that this result was due to a single gene within or associated with the *B* system. The incidence of metastatic tumors and mortality of individuals infected with Rous sarcoma virus was lower in B^2B^2 than B^5B^5 haplotypes (Collins et al., 1986). New Hampshire chickens with the $B^{24}B^{24}$ haplotype regressed tumors more rapidly than $B^{23}B^{23}$ or $B^{23}B^{24}$ individuals, despite an interaction of haplotype with dosage of virus (Vincent and Taylor, 1988).

Resistance to coccidiosis is also affected by haplotype at the *B* system (Hartmann et al., 1985). Chickens of B^2B^2 haplotype had significantly higher lesion scores to Eimeria tenella than did those of haplotypes B^2B^5 or B^5B^5 (Clare et al., 1985). A line selected for resistance to E. tenella had different haplotypes at the *B* system than the accompanying line which was selected for susceptibility (Johnson and Edgar, 1986). Inbred lines differing at the MHC differed in resistance to E. tenella, E. acervulina, and E. maxima. In all cases the line with $B^{15}B^{21}$ was more resistant than the one with B^2B^2 (Lillehoj and Ruff, 1986).

Other alloantigen systems. While the *B* complex is a major system affecting disease resistance, other erythrocyte alloantigen systems in-

fluencing resistance to infectious agents have been identified in chickens. Allelic differences at the *A-E* linked loci were observed in chickens selected for resistance and susceptibility to cecal coccidiosis. The resistant line had high frequencies of haplotypes A^6-E^5 and A^9-E^1 while the susceptible line did not carry those haplotypes, but did carry haplotype A^{11}-E^1, which was not present in the resistant line (Johnson and Edgar, 1984).

The *C* locus, a minor histocompatibility locus (Schiermann and Nordskog, 1965), is as yet not well studied. Johnson and Edgar (1986) studied lines selected for high or low resistance to *E. tenella*, and found that frequency of alleles at the *C* locus remained quite stable during selection.

There were indications that alleles at the *I* locus contribute to variation within lines in coccidiosis response. In both natural and controlled exposures to *E. tenella*, I^4I^4 chickens were more resistant than I^2I^4 or I^2I^2 individuals (Martin et al., 1986). This result was supported by Johnson and Edgar (1984), who observed correlated responses in frequency of alleles at the *I* locus to selection for and against resistance to *E. tenella*.

Dwarfism. Interest has been generated in the effects of other genes on immune responses, particularly those of the sex-linked dwarf locus (*dw*), first described by Hutt (1959). In mice, dwarfs have lower antibody response than non-dwarfs (e.g. Duquesnoy, 1972). Marsh (1983) observed lower titers to sheep red blood cells (SRBC) in dwarf White Leghorns than

non-dwarfs and postulated that this difference was due to lower triiodothyronine (T_3) levels in the former. Differences in serum levels of thyroid hormones of dwarf and normal chickens have been reported for both meat- and egg-type chickens, and were attributed to decreased peripheral conversion of thyroxine (T_4) to T_3 (May and Marks, 1983; Scanes et al., 1983).

Attempts to alter immune responses by changing thyroid hormone levels have yielded mixed results. Feed supplementation with T_4 increased serum concentrations of T_3 and T_4 and thymus weights in both dwarf and normal White Leghorns (Marsh et al., 1984a; Bachman and Mashaly, 1986). Bursa weight was increased in one study (Marsh et al., 1984a) but was not changed in another (Bachman and Mashaly, 1986). In neither study was there an effect on antibody response to SRBC. Feed supplementation with T_3 had differential effects in dwarf and normal chickens. In normal chickens, serum T_3 and antibody response to SRBC were not changed; bursa weight remained the same in one study (Bachman and Mashaly, 1986) and increased in another, along with thymus weight (Marsh et al., 1984b). In dwarf chickens, serum T_3 and thymus weight increased, and bursa weight and SRBC antibody response decreased (Marsh et al., 1984b).

Circulating concentrations of thyroid hormones can be reduced by chemical means or through thyroidectomy. White Leghorns fed thiouracil had higher primary antibody titers to SRBC and greater persistence of titers than control chicks. The secondary antibody response, while lower for thiouracil-fed than for control males, did not differ for females (Scott et al., 1985). Yam et al. (1981) observed lower titers to SRBC

in thiouracil-fed chicks than in controls, chicks fed T_4, or chicks fed thiouracil and T_4. Primary and secondary antibody responses to SRBC or Brucella abortus were not affected by hypothyroidism caused by thyroidectomy or by feeding thiouracil (Mashaly et al., 1983; Bachman and Mashaly, 1986).

When thiouracil was fed, a reduction in body weight was observed (e.g. Scanes et al., 1984) as well as a reduction in plasma T_3 and T_4. It is unknown whether this reduction in body weight was due to deficiency of T_3 or of T_4, since T_4 is converted to T_3 in the body. Supplementation with T_4 (15 μg/kg body weight injected daily), however, restored normal body and skeletal growth in thyroidectomized chicks and almost normal growth in chicks fed thiouracil (Scanes et al., 1984). Although thyroid hormones are necessary for growth, T_4 administered at .10 to 10 ppm in the diet to chicks with normal thyroid hormone levels had little effect on growth rate or T_3 serum concentrations, while dietary T_3 suppressed growth in normal broilers at higher (1.00 ppm) but not lower (.05 to .25 ppm) dosages (May, 1980).

Huybrechts et al. (1986) indicated that T_3 and T_4 levels of dwarf chickens differed from non-dwarfs only at young ages (2-9 weeks). These results were supported by those of Callahan and Parsons (1986) who found lower T_3 levels in dwarf than non-dwarf meat-type chickens at 2 weeks but not between 4 and 10 weeks of age. No differences in antibody response to Newcastle or infectious bursal disease vaccines, or to SRBC were found between dwarf and nondwarf broiler breeder females (Lilburn et al.,

1986b), indicating that such responses differ between dwarf meat-type and egg-type chickens.

Response differences between normal and dwarf chickens could be masked when given high dosages of SRBC antigen (Marsh, 1983). Similar results were observed when lines of chickens selected for high and low antibody response to SRBC were given varying doses of that antigen (Ubosi et al., 1985b). Dosage and route of administration of SRBC may have contributed to differences in results of trials with dwarf chickens.

Chickens selected for high and low body weight at 8 weeks of age differed in thyroid hormone profiles in an age-dependent manner, with the high weight line chicks having lower T_3 and higher T_4 concentrations at 61 but not 25 days of age (Nir et al., 1987). The sex-linked allele for dwarfing was incorporated into these lines through repeated backcrosses, creating sublines of dwarf and non-dwarf individuals. Experiments using these birds revealed a line x dwarf genotype interaction for antibody response to SRBC (Mauldin et al., 1978). Chicks from the low weight line had higher titers than those from the high weight line, but the high weight dwarfs had higher titers than non-dwarfs, while the low weight dwarfs had lower titers than normals. The low antibody response of high weight chickens is similar to the generally low immunoresponsiveness of broilers (Siegel et al., 1984; Dunnington et al., 1987).

Selection for general immunity

Antibody response to sheep erythrocytes. Lines of mice selected divergently for antibody production to SRBC were developed in two se-

lection experiments (Biozzi et al., 1970a; Feingold et al., 1976). In the first experiment, lines were selected for response to SRBC for the first 8 generations. Thereafter the selection criterion was changed because of effects of maternal antibodies on the offspring's titers, and the lines were selected for response to sheep or pigeon erythrocytes in alternate generations. Titer levels plateaued after 13 generations of selection (Biozzi et al., 1970a), with realized heritabilities of .25 and .33 in the upward and downward directions, respectively (Feingold et al., 1976). In generation 17 of selection there was a 200-fold difference between lines for antibody titers to SRBC and a 100-fold difference to pigeon erythrocytes. When tested for antibody response to several unrelated antigens, in all cases more antibody was produced by the high than the low responder line (Biozzi et al., 1972). Antigen processing by macrophages also differed between lines for a variety of antigens tested (Biozzi et al., 1982). Serum IgM, IgA, and IgG levels were greater in the high than low line, both prior to and following inoculation with SRBC or pigeon erythrocytes (Biozzi et al., 1970b).

In a second selection experiment for high and low antibody response to SRBC in mice, the interval between weaning and injection with sheep erythrocytes was lengthened to avoid confounding by maternal antibody (Feingold et al., 1976). A selection limit was attained in 14 generations, with 83-fold differences between lines, and realized heritabilities of .22 and .18 in the upward and downward directions, respectively (Feingold et al., 1976).

Antibody response to SRBC was polygenic in both selection experiments, with the number of involved loci estimated at 7 to 12 (Feingold et al., 1976). Also involved were major histocompatibility complex haplotypes, which differed greatly between lines (Colombani et al., 1979), and accounted for 12 to 20% of the variability in antibody response to the selected dose (Mouton et al., 1979).

When guinea pigs were selected for high or low responses to sheep and chicken RBC (given in alternate generations) changes were also observed in responsiveness to Salmonella typhimurium antigens (Ibanez et al., 1980). A realized heritability of .18 was obtained after 8 generations of selection on the difference between selected lines.

Chickens responded to selection for high and low antibody response to SRBC with realized heritabilities after 4 generations of selection of .20 to .30 in the upward and .22 to .52 in the downward directions (Siegel and Gross, 1980; van der Zijpp et al., 1988). Such lines differed in resistance to a variety of infectious agents including Mycoplasma gallisepticum, Newcastle disease, splenomeglia virus, Staphylococcus aureus. Escherichia coli. Eimeria necatrix (Gross et al., 1980), Eimeria tenella, (Martin et al., 1986), and Marek's disease (Dunnington et al., 1986; van der Zijpp et al., 1988). Line differences were also noted for resistance to certain neurotoxic chemicals (Ehrich et al., 1986; Dunnington et al., 1989) and antigens such as bovine serum albumen, Brucella abortus, and Salmonella H antigen (van der Zijpp and Nieuwland, 1986). Moreover, these lines differed in ontogeny (Ubosi et al., 1985a) and kinetics of response to primary and secondary immunizations with SRBC

(Ubosi et al., 1985b). Magnitude of genetic differences was also modified by environmental factors such as dosage (van der Zijpp, 1983b; Ubosi et al., 1985b), immunization history (van der Zijpp et al., 1982), and route of administration (van der Zijpp et al., 1986).

Correlated responses in growth and reproductive traits resulted from divergent selection for antibody response to SRBC. Juvenile body weights were lower in chickens from lines selected for high antibody response (Siegel et al., 1982; van der Zijpp et al., 1987) and these differences persisted until 24 weeks of age. Age at first egg was later in the line selected for high antibody response (Siegel et al., 1982; Dunnington et al., 1984).

The Virginia high (HA) and low (LA) antibody response lines to SRBC differed in allelic frequencies at the *A, B, C, D, E,* and *H* erythrocyte alloantigen loci, but not loci *I* or *L* (Dunnington et al., 1984). Lines were almost completely divergent for haplotypes at the *B* complex, with line HA 61% homozygous for B^{21} and line LA 98% homozygous for B^{13} in generation 10. Divergence at the *B* complex continued, with line HA >90% homozygous for B^{21} in generation 12 (Dunnington et al., 1986). Differences in susceptibility to Marek's disease (Dunnington et al., 1986) and Eimeria tenella (Martin et al., 1986) were reported for these lines, but because of confounding of line and *B* haplotype no conclusions could be drawn as to which was responsible for these differences in resistance. Similarly, lines divergently selected from ISA Warren chickens for SRBC titers differed in resistance to Marek's disease (van der Zijpp et al., 1988), and frequency of B^{21} was greater in the line selected for high

titers than low titers after 4 generations of selection (van der Zijpp et al., 1987).

Single gene selection. Eight chicken sublines were developed (Kim et al., 1987) from a White Leghorn line. These 8 lines had all possible combinations of 3 traits: 2 haplotypes at the MHC (B^1B^1 or $B^{19}B^{19}$); antibody response to GAT, a polymer of glutamic acid, alanine and tyrosine (GAT-HI or GAT-LO); and were either progressors or regressors of induced tumors to Rous sarcoma virus. Antibody response to GAT is controlled by a gene linked to the MHC (Pevzner et al., 1978). Sublines differed in response to Pasteurella multocida (Lamont et al., 1987) and Marek's disease (Steadham et al., 1987), with those carrying B^{19} showing greater resistance. Antibody response to a P. multocida vaccine, however, was not affected by B haplotype (Cheng and Lamont, 1988). GAT-HI lines were more resistant to Marek's disease than GAT-LO lines, but Rous sarcoma response type did not affect resistance (Steadham et al., 1987). Antibody response to a M. gallisepticum vaccine was higher in B^1 than B^{19} and in GAT-HI than GAT-LO lines (Cheng and Lamont, 1988).

Phagocytosis in these sublines was measured by a carbon clearance assay, and T-cell mediated immunity was measured by wing-web swelling in response to phytohemagglutinin injection. Line x sex interactions were evident in these responses, with phagocytic responses being greater in GAT-HI than GAT-LO females, and in B^1 than B^{19} males. Similarly, females did not differ for wing-web swelling, while in males responses were greater in B^1 than B^{19} lines and Rous sarcoma regressor than progressor lines (Cheng and Lamont, 1988). Differences between sexes and B

haplotypes for phytohemagglutinin wattle response have been reported elsewhere (Taylor et al., 1987).

Rous sarcoma response type did not affect juvenile body weight or antibody response to SRBC. The interaction between GAT type and *B* haplotype was significant for these traits. Within the GAT-HI lines *B* haplotypes did not differ, but the GAT-LO lines carrying B^{19} had lower juvenile body weight and SRBC antibody titers than those with B^{1} (Kim et al., 1987).

Lymphoid organs. Lines of chickens were developed to explore the effects on immune response of increased and decreased size of the bursa of Fabricius (Yamamoto and Glick, 1982), one of the two lymphoid organs of chickens (Glick et al., 1956). The line selected for small bursa size produced more antibody to SRBC in both primary and secondary responses. Of this response, proportionally more was due to IgG in the primary response of the small bursa line, but this was not so for either IgM or for the secondary response. Total (nonspecific) serum IgG was greater in the large than small bursa line before and after both primary and secondary responses to SRBC (Yamamoto and Glick, 1982). When antibody responses to bovine serum albumen and dinitrophenyl were measured, primary response was similar for both lines. After a secondary inoculation, however, titers were higher at 7 but not 14 or 21 days post inoculation in the small bursa line (Yamamoto and Glick, 1982).

Differential immune responses. Divergent selected lines have been established for several immunological responses, including circulating levels of IgG, graft-versus-host (GVH) reactions (with two sublines for

different *B* haplotypes), antibody response to rabbit serum albumen, and anaphylactic shock to bovine serum albumen (Okada and Yamamoto, 1987; Yamamoto et al., 1988). When selection was for high or low circulating IgG, the high line had greater antibody response to SRBC and lipopolysaccharide (LPS), GVH response, susceptibility to Marek's disease and resistance to coccidiosis than the low IgG line (Okada and Yamamoto, 1987; Yamamoto et al., 1988; Tamaki et al., 1988).

The line selected for high antibody response to rabbit serum albumen had greater titers to SRBC than the low line, but did not differ in response to bovine serum albumen (BSA) or a Marek's disease challenge (Yamamoto et al., 1988). Similarly, the line selected for high anaphylactic shock to BSA had greater antibody responses to BSA than the low line, but did not differ for SRBC antibody or Marek's resistance (Yamamoto et al., 1988). Within the lines selected for high or low GVH response there were no differences in antibody response to SRBC, BSA, or LPS. There was a *B* haplotype x GVH response interaction for resistance to Marek's disease in these lines. Lines carrying B^9 did not differ in resistance to Marek's, while of the lines carrying B^{11} that with high GVH response was more susceptible (Okada and Yamamoto, 1987; Yamamoto et al., 1988). The identification of *B* haplotypes in these reports is not consistent with those routinely used (Briles et al., 1982b).

Divergent selection for an index of antibody response to Newcastle disease virus and E. coli at a young age (10 and 18 days, respectively) resulted in divergence between lines for response to both antigens (Pitcovski et al., 1987). The high line had higher titers to both

antigens at several ages tested, and the ontogeny of response to Newcastle virus differed considerably between the lines. The proportion of non-responders to E. coli at 18 and 22 days of age was reduced in the high line. Correlated responses included lower body weight at 7 weeks, fertility and semen volume in low than high titer index line, and no differences between lines in hatchability of fertile eggs or spermatocrit (Pitcovski et al., 1987).

Immunoglobulins

Mercaptoethanol-resistant (MER) antibodies are a commonly used measure of immunoglobulin G (IgG) response in chickens, while ME-sensitive (MES) titers consist primarily of IgM (Delhanty and Solomon, 1966). IgG is the major portion of an antibody response in higher animals, and differs from IgM in ability to neutralize viral infectivity, bacterial immunity, interactions with other immune components, and serological reactions, including hemagglutination (Barrett, 1983). After treatment with 2-ME, chicken antibodies to Salmonella gallinarum had only about 10% their previous bactericidal activity (Solomon, 1968).

In chickens, genetic differences have been reported for MER production in response to SRBC inoculation. Yamamoto and Glick (1982) observed higher total and MER primary antibody titers in the small than large bursa line. Comparing White Plymouth Rock, ISA Warren, and White Leghorn stocks of chickens, appearance of MER antibodies 3 days post primary inoculation (PPI) was limited to the White Plymouth Rocks. By 7 and 14 days PPI, however, MER antibodies were present in all 3 stocks (van

der Zijpp, 1983b). Chickens from lines selected for high and low primary antibody response to SRBC differed in MER response to a secondary inoculation with SRBC (van der Zijpp and Nieuwland, 1986).

In a study using lines HA and LA (Ubosi et al., 1985b) the absolute MER response in primary titers was greater in line HA than LA, but was proportionately similar (approximately 50% of the total antibody) in both lines. Phenotypic correlations of total with MER and MES antibodies, respectively, were .70 and .94, for primary response. In White Leghorn chicks inoculated intramuscularly (i.m.) with SRBC, little MER was present 3 days PPI (van der Zijpp and Leenstra, 1980). There was then a sharp rise in MER levels to 7 days PPI, with about 60% MER, which continued to increase gradually to about 75% on day 13. Primary antibody responses to SRBC of White Plymouth Rocks injected i.m. were almost completely MES antibody; MER antibody titers were very low 3, 5, 7 or 10 days PPI (van der Zijpp et al., 1983).

MER antibodies made up about 30% of the response 3 days post secondary inoculation (PSI) with SRBC, and about 50% of the response 5, 7, and 10 days PSI (van der Zijpp et al., 1983). Yamamoto and Glick (1982) found that MER antibodies constituted about 50 to 70% of the secondary response to SRBC in their large and small bursa lines, with no differences between lines. Ubosi et al. (1985b) reported that although absolute levels of MER for both lines were similar PSI, MER was about 54 and 68% of total antibody in lines HA and LA, respectively. Phenotypic correlations of total with MER and MES antibodies for secondary response were .43 and .79, respectively.

Vaccination had a marked effect on both total and MER titers to SRBC in White Leghorn chickens (van der Zijpp et al., 1982). Vaccination against Marek's, Newcastle, and infectious bursal diseases enhanced total SRBC titers. In the vaccinated group the response curve for MER was altered, being lower 4 days PPI, similar 7 days PPI, and higher 13 days PPI than the non-vaccinated group. The MES titers were higher for vaccinated chicks at all times measured.

Differences between genetic stocks in MER responses may be due, in part, to differences at the MHC. Circulating levels of IgG and IgM are controlled by both MHC-linked (Rees and Nordskog, 1981) and MHC-independent genes (Ivanyi and Lydyard, 1975). Although genetic control of IgG and IgM levels following an immune response has not been determined, Lillehoj and Ruff (1986) reported differences in IgM and IgA among inbred lines of chickens differing at the MHC following infection with several different strains of Eimeria.

CHAPTER I

IgG AND IgM RESPONSES OF CHICKENS SELECTED FOR HIGH OR LOW ANTIBODY RESPONSE TO SHEEP ERYTHROCYTES

Introduction

Responses to genetic selection for high and low antibody titers to sheep red blood cells (SRBC) have been shown in mammals and birds (Biozzi et al., 1970b; 1971; Ibanez et al., 1980; Siegel and Gross, 1980; van der Zijpp et al., 1987). Experiments with two such lines of White Leghorn chickens have elucidated the kinetics of these responses for the high (HA) and low (LA) response lines and crosses between them (Ubosi et al., 1985b). Lines HA and LA also differed in resistance to infectious diseases, including *Mycoplasma gallisepticum*, Newcastle Disease, Escherichia coli, Staphylococcus aureus (Gross et al., 1980), Eimeria tenella (Martin et al., 1986), and Marek's disease (Dunnington et al., 1986), as well as reproductive traits, body weight and feed efficiency (Siegel et al., 1982; Chapter IV)

2-Mercaptoethanol-resistant (MER) antibodies are a commonly used measure of IgG response in chickens, while 2-ME-sensitive (MES) titers consist primarily of IgM (Delhanty and Solomon, 1966). IgG is the major portion of an antibody response, and differs from IgM in ability to neutralize viral infectivity, bacterial immunity, interactions with other immune components, and serological reactions, including hemagglutination (Barrett, 1983). After treatment with 2-ME, antibodies to Salmonella gallinarum in chickens had only about one-tenth their previous bactericidal activity (Solomon, 1968).

Although correlated responses of unselected traits to selection for antibody titers to SRBC have been reported, information on IgG and IgM

is generally lacking. In mice selected for high and low antibody response to sheep and pigeon red blood cells, serum levels of IgG, IgM and IgA were greater in the high than the low responder line, both prior to and following inoculation with sheep or pigeon erythrocytes (Biozzi et al., 1970b). Since relative amounts of MER and MES change over the course of the immune response (van der Zijpp et al., 1983), the experiment reported here was conducted to determine the kinetics of IgG and IgM in antibody response of lines of chickens selected differentially for total antibody titer to SRBC.

Materials and Methods.

Stocks and hemagglutination assays

Chicks used in this experiment were from 14th generation matings of two lines (HA and LA) selected divergently for antibody response 5 days post inoculation with .1 ml of a .25% suspension of sheep erythrocytes (Siegel and Gross, 1980). Because this dose resulted in maximal separation of titers in these lines (Ubosi et al., 1985b), it was used throughout this experiment. Within each trial chicks from each line were obtained from age-contemporary parents.

Total SRBC antibody titers were measured by a microtiter hemagglutination procedure (Wegmann and Smithies, 1966). MER titers were determined by incubating plasma with .15 *M* 2-mercaptoethanol (2-ME) at 37°C for 30 minutes prior to dilution for determination of titers (Delhanty and Solomon, 1966). MES was defined as the difference between total and MER antibody titers. All titers are expressed as $\log_2$ of the reciprocal of the highest dilution in which there was hemagglutination.

Trial 1

A preliminary experiment was conducted to compare lines HA and LA for MER and MES titers. Twenty chicks from each line were vaccinated against Marek's disease and raised in battery brooders with wire floors, continuous lighting, and food and water available ad libitum. At 21 days of age chicks were injected intravenously with SRBC and a random sample

of half of the chicks from each line was bled either at 3 or 5 days post primary inoculation (PPI) and their antibody titers determined.

Trial 2

Sixty chicks per line were immunized against Marek's disease at hatch and reared in battery brooders with ad libitum feed and water and constant lighting. At 31 days of age chicks were inoculated intravenously with SRBC and antibody titers measured in a sample of 12 to 19 chicks per line 3, 5, 7 and 12 days PPI. Twenty-four days PPI half of the chicks were given a booster dose of SRBC. All chicks were bled; half of the inoculated and uninoculated chicks were bled 3 days and half 5 days post-secondary inoculation (PSI) for determination of antibody titers.

Estimates of genetic correlations of total antibodies with MER and MES 5 days PPI were obtained using the equation

$$r_{GG'} = \frac{\Delta G'}{\Delta G} \frac{h}{h'} \frac{\sigma P}{\sigma P'}$$

where ΔG and $\Delta G'$ are changes due to selection in the selected and unselected traits, respectively, h and h' are respective square roots of heritabilities, and σ_P and $\sigma_{P'}$ are the respective phenotypic standard deviations (Falconer, 1954). In this case the selected trait is total titers to SRBC and the unselected are MER and MES. The change in titers due to selection was calculated as the difference between the selected lines; realized heritability for total titers was .26, the mean value reported for these lines (Siegel and Gross, 1980). Since heritabilities for MER and MES were not available for these lines, average heritability

estimates (.14 for MER and .34 for MES) from unselected White Leghorns 7 days PPI were used (van der Zijpp and Leenstra, 1980).

Statistical analyses

Analyses of variance were conducted to determine effects of line and days PPI or PSI on total, MER, and MES titers, as well as % MER (i.e., the proportion of the total antibody titer due to MER). When significant differences were found among days, Duncan's multiple range test was conducted to differentiate among means.

Results

Trial 1

(Preliminary Trial). Line x day PPI interactions were significant for total titers and MER, but not for MES. To determine the cause of the interactions, analyses of variance were conducted within lines and within days PPI. Interactions for both total and MER titers were due to a lack of difference between lines 3 days PPI, but higher total and MER titers for HA than for LA chicks 5 days PPI. Total titers 5 days PPI were 7.0 ± 3.2 and 2.3 ± 1.9 for lines HA and LA, respectively, and corresponding MER titers 5 days PPI were 5.3 ± 3.5 and 1.7 ± 1.8. Lines did not differ for MES titers, nor did MES change from days 3 to 5 PPI.

Trial 2

Line x day PPI interactions were significant for all titers. Again, to further elucidate genetic and kinetic effects, analyses of variance were conducted within lines and days PPI. Line HA chicks had higher total antibodies than those from line LA at all times measured (Table 1), thus, interactions were due to differences in the response curves of the selected lines; that is, both started at zero but values for line HA reached higher levels than those for line LA. In line HA, primary titers were lowest 3 days PPI, peaked at 7 days, and declined between day 7 and 12, with a further decrease to somewhat low residual titers at 27 and 29 days PPI. Although line LA chicks followed a similar trend, total titers were considerably lower with no difference between days 5 and 7.

Five days PPI, levels of MER and MES were higher in HA than LA chicks, a pattern that persisted throughout the trial (Table 1). Within line HA, MES was highest 5 days PPI and then declined. In line LA, however, after an initial rise MES levels plateaued through at least 12 days PPI. Peak MER titers were observed 7 days PPI in line HA while in line LA MER remained low at all times. The differential temporal response of the lines was reflected in the proportion of MER to total titers, where % MER was much lower in line LA than HA by 5 days PPI. Percentage MER was greatest 7 days PPI in both lines, after which it quickly subsided to near zero in line LA, while persisting at modest levels in line HA to 29 days PPI. MER was never greater than 20% of the total response in line LA. Realized genetic correlations of total titers with MER and MES 5 days PPI were .33 and .59, respectively.

Line x day PSI interactions were significant for total, MER and MES antibody. Comparisons between lines showed higher total and MER titers for HA than LA chicks (Table 2). Both on an absolute and on a relative basis there was a greater increase in total titers between days 3 and 5 PSI for line LA than HA. Although this pattern existed for MER on a relative basis, the absolute change was greater in line HA than LA, with titers 3 days PSI for line HA similar to 5 day PSI titers for line LA. MES was higher 3 days and lower 5 days PSI in line HA than LA. This decrease in MES in line HA was accompanied by the marked increase in MER.

Discussion

Selection for total antibody response to SRBC acted in the same direction on both MER and MES. These correlated responses agreed with those observed in mice (Biozzi et al., 1970b). Moreover, the somewhat earlier peak observed for IgM than IgG is typical (Barrett, 1983).

The % MER 5 days PPI was somewhat higher in line HA and lower in line LA than previously found for these lines (Ubosi et al., 1985b), a result which is not surprising because of the 5 additional generations of selection. Studies using other populations have reported values 5 or 7 days PPI ranging from 5% MER (van der Zijpp et al., 1983) to 65 % MER (van der Zijpp and Leenstra, 1980; van der Zijpp et al., 1982), a range consistent with our findings.

Secondary responses were expected to consist primarily of IgG (Barrett, 1983), and results for line HA followed this pattern. In line LA, however, both primary and secondary titers were largely MES antibodies. Although selection for low antibody production acted against both IgG and IgM, the one more conserved was IgM, the more primitive antibody response (Barrett, 1983). Higher MER titers PSI than PPI were consistent with previous results with these lines (Ubosi et al., 1985b), as well as with those with other populations of chickens (van der Zijpp et al., 1983; Yamamoto and Glick, 1982).

Genetic differences between stocks of chickens have been reported for MER production in response to SRBC inoculation. Yamamoto and Glick (1982) observed higher total and MER primary antibody titers in a line

selected for small than for large bursa size. When lines were compared for secondary antibody responses, again total titers were greater for the small than the large bursa line. No line difference was present, however, for MER or MES in secondary responses (Yamamoto and Glick, 1982). Bursa size is smaller for LA than HA chicks (Ubosi et al., 1985a). Comparing White Plymouth Rock, ISA Warren, and White Leghorn stocks of chickens, appearance of MER antibodies 3 days PPI was limited to the White Plymouth Rocks. By 7 and 14 days PPI, however, MER antibodies were present in all 3 stocks (van der Zijpp, 1983b).

Differences between lines in MER and MES responses to SRBC suggest that selection for total antibody response has altered the number of antibody-producing cells. Such differences may be due, in part, to differences at the major histocompatibility complex (MHC). In generation 10, line HA had 80% B^{21} at the MHC (Dunnington et al., 1984) while line LA had 98% B^{13}. The B^{21} haplotype confers resistance to Marek's disease, a neoplastic disease, while B^{13} imparts susceptibility (Briles et al., 1980), though such resistance or susceptibility may depend upon the background genome in which the haplotypes are found (Hartmann et al., 1986; Hartmann, 1988). By the 14th generation the respective gene frequencies were 98% in each line. Circulating levels of IgG and IgM are controlled by both MHC-linked (Rees and Nordskog, 1981) and MHC-independent genes (Ivanyi and Lydyard, 1975). Although genetic control of IgG and IgM levels following an immune response has not been determined, differences in IgM and IgA exist among inbred lines of chickens differing at the MHC following infection with different strains of Eimeria

(Lillehoj and Ruff, 1986). Negative genotypic correlations between MER and MES responses to SRBC were reported by van der Zijpp and Leenstra (1980).

The part-whole genetic correlations of total titers with MER and MES titers 5 days PPI are consistent with the greater differences between lines for MES than MER. Thus, while selection for increased antibody response to SRBC antigens would modify both IgG and IgM in a positive direction, the change would not be symmetrical and could be further complicated by changes in the IgM to IgG switch. Since MER plus MES equal the whole, the difference between them would become greater, as evidenced by a negative correlation between them (van der Zijpp and Leenstra, 1980). This negative correlation is consistent with the thesis that natural selection probably favors some form of an intermediate optimum for antibody production.

Summary

Kinetics of IgG and IgM as measured by 2-mercaptoethanol resistant (MER) and susceptible (MES) antibodies to sheep erythrocytes, respectively, were determined as correlated responses in lines of chickens selected for high (HA) and low (LA) antibody response to sheep erythrocytes. Primary response patterns for total, MER and MES antibody differed according to genetic line. Total antibody increased rapidly, peaked, and persisted at moderate levels in line HA while both peak and persistency were lower in line LA. Levels of MES peaked and then declined in line HA chickens but persisted at low levels in line LA. Titers of MER antibody were considerably greater in line HA than LA, on both an absolute basis and as a proportion of total antibody titer. Secondary total titers were greater 5 than 3 days after injection and for line HA than LA chicks. The pattern observed for MER and MES in line LA was similar to that for total antibody, as was MER in line HA. For MES the pattern was reversed in line HA.

Table 1. Means and standard deviations for total, 2-mercaptoethanol-resistant (MER) and -susceptible (MES) primary antibody titers to sheep erythrocytes in lines selected for high (HA) and low (LA) antibody response (Trial 2).

		Days PPI					
Titer	Line	3	5	7	12	27	29
Total	HA	2.1 ± 1.5 e	9.9 ± 1.2 b	11.4 ± 1.2 a	7.2 ± 1.8 c	3.7 ± 1.2 d	4.5 ± 1.5 d
		**	**	**	**	**	**
	LA	0.3 ± 0.8 c	2.8 ± 1.6 ab	3.9 ± 3.7 a	2.3 ± 2.1 b	0.2 ± 0.6 c	1.7 ± 2.1 bc
MES	HA	2.1 ± 1.5 c	7.8 ± 1.4 a	4.6 ± 2.7 b	4.7 ± 2.0 b	3.3 ± 1.5 bc	3.4 ± 1.2 bc
		NS	**	**	**	**	**
	LA	0.3 ± 0.0 b	2.7 ± 1.7 a	2.9 ± 3.1 a	2.2 ± 2.0 a	0.2 ± 0.6 b	1.7 ± 2.1 a
MER	HA	0.0 ± 0.0 d	2.2 ± 1.2 bc	6.8 ± 3.0 a	2.6 ± 1.8 b	0.4 ± 1.2 d	1.1 ± 1.0 cd
		NS	**	**	**	NS	**
	LA	0.0 ± 0.0 b	0.1 ± .3 b	1.0 ± 2.1 a	0.1 ± 0.2 b	0.0 ± 0.0 b	0.0 ± 0.0 b
% MER	HA	0	21	59	37	10	22
	LA	0	4	18	1	0	0

** indicate differences ($p \leq .01$) between selected lines.
a,b indicate differences ($p \leq .05$) between titers within a row.

Table 2. Means and standard deviations for total, 2-mercaptoethanol-resistant (MER) and -susceptible (MES) antibody titers to a secondary inoculation with sheep erythrocytes in lines selected for high (HA) and low (LA) antibody response (Trial 2).

Titer	Line	Days PSI 3	5
Total	HA	7.7 ± 1.1 b **	11.4 ± 1.3 a **
	LA	2.2 ± 1.6 b	7.9 ± 3.0 a
MES	HA	5.6 ± 0.9 a **	3.1 ± 2.0 a **
	LA	2.1 ± 1.6 a	5.3 ± 1.9 a
MER	HA	2.1 ± 1.2 b **	8.3 ± 1.4 a **
	LA	0.1 ± 0.5 b	2.6 ± 2.7 a
% MER	HA	27	74
	LA	4	27

** indicate differences ($p \leq .01$) between selected lines.
a,b indicate differences ($p \leq .05$) between titers within a row.

CHAPTER II

MAREK'S DISEASE AND MHC HAPLOTYPES IN CHICKENS FROM A LINE SELECTED FOR LOW ANTIBODY RESPONSE TO SHEEP ERYTHROCYTES

Introduction

The major histocompatibility complex (MHC) of the chicken is located on a microchromosome (Bloom and Bacon, 1985) and consists of three regions, *B-L*, *B-F*, and *B-G* (Hala et al., 1977; Pink et al., 1977). *B-L* region codes for class II MHC antigens, *B-F* codes for class I and class IV antigens, and *B-G* codes for class IV antigens (Vainio et al., 1987). Class IV antigens on erythrocytes allow determination of MHC haplotypes in chickens through serotyping of the *B* alloantigens.

The *B*-complex, as the MHC in chickens is known, affects resistance to diseases in chickens, most notably neoplastic diseases. The strongest documented association is that between the MHC haplotypes and resistance to Marek's disease (e.g., Briles et al., 1977; 1980; 1982a), which has been mapped to the *B-F* subregion of the MHC (Briles et al., 1983). *B* haplotypes are also associated with resistance or susceptibility to several other diseases and antigens (see review by Bacon, 1987).

Correlated responses to selection of chickens for high (HA) and low (LA) antibody response to sheep erythrocytes (Siegel and Gross, 1980) included gene frequency at the MHC (Dunnington et al., 1984), production traits (Siegel et al., 1982), and disease resistance (Gross et al., 1980; Martin et al., 1986), including Marek's disease (Dunnington et al., 1986). Gene frequencies at the MHC in the 10th generation of selection were 80% B^{21} in line HA and 99% B^{13} in line LA (Dunnington et al., 1984). The B^{21} haplotype is noted for its considerable resistance to Marek's disease (Longenecker et al., 1976; Briles et al., 1977; 1980) while B^{13} confers

moderate susceptibility to Marek's (Briles et al., 1980; Schat et al., 1981). It was unknown whether differences between lines HA and LA were due to the MHC or to the background genome.

The combination of haplotypes at the MHC can be nearly as important in resistance to Marek's disease as the particular haplotypes present (Hartmann et al., 1986). Non-MHC genes may also play an important role in resistance (Pevzner et al., 1981; Fredericksen et al., 1982). We have developed a series of sublines from lines HA and LA differing at the MHC. Reported here are comparisons of mortality from Marek's disease of chickens differing in MHC haplotypes within line LA.

Materials and Methods

Stocks

Lines HA and LA were derived from Cornell Randombred White Leghorns through divergent selection for antibody response 5 days after inoculation with 0.1 ml of 0.25% sheep erythrocytes (Siegel and Gross, 1980). In the 12th generation of selection, gene frequencies at the MHC were 96% B^{13} and 96% B^{21} in lines LA and HA, respectively. These two haplotypes are the only ones now segregating in these lines, and serotyping has confirmed that the *B-F* and *B-G* subregions of B^{13} and B^{21} differ in these lines. In the 12th generation of selection, sublines of HA and LA were developed to provide chickens of the three *B* genotypes within each background genome (Dunnington et al., 1988). Backcross matings of line LA background $B^{13}B^{21}$ parents were such that 87.5% of the background genome of chicks used in this experiment was derived from line LA.

Husbandry

Chicks were removed from the hatcher, wingbanded and placed in floor pens containing wood shaving litter from a previous flock. Feed and water were provided ad libitum throughout the experiment. Lighting was continuous until 63 days of age and a light:dark ratio of 16:8 h was maintained thereafter. At 17 days of age all chicks were bled and typed at the *B* complex. Body weights were obtained at 28 days of age. Mortality was recorded daily to 126 days of age, and all birds were autopsied to

determine sex and cause of death. Macroscopic lesions due to Marek's disease were classified as visceral and/or neural.

Statistical analyses

Mortality rates were compared by the Lee-Desu test (Hall and Nie, 1981) and cumulative mortality was compared by a modified chi-square procedure designed to test for effects of single alleles and allelic interactions on disease liability (Norwood and Hinkelmann, 1978). Differences in cumulative mortality between sexes and differences in incidence of neural and visceral lesions between sexes and among haplotypes were tested by chi-square. Age at which death from Marek's disease occurred was tested by analysis of variance for sex, sire, dam and MHC haplotype main effects and interactions. Body weights were transformed to natural logarithms and analysis of variance was conducted to determine if differences between sexes and MHC haplotypes existed. Differences presented in the text are significant at $p \leq .05$.

Results

Blood typing at the *B* complex resulted in the expected 1:2:1 ratio of haplotypes, with 51 $B^{13}B^{13}$, 111 $B^{13}B^{21}$ and 37 $B^{21}B^{21}$ individuals. Body weights at 28 days of age were greater for males than females (Table 1), but there were no significant differences among haplotypes or for the sex x haplotype interaction. Seven chicks died prior to the onset of Marek's disease (3.5% mortality); 3 were $B^{13}B^{13}$, 4 were $B^{13}B^{21}$.

Cumulative mortality due to Marek's disease was 56%, with mortality rate and total mortality greater for females than males (Figure 1). Mortality commenced at a younger age in females than males (Table 1), with mean age at death also earlier for females. There was no interaction between sex and B haplotypes for age at death. There were no differences among haplotypes for mortality rate (Figure 2), nor was cumulative mortality affected by individual haplotypes or haplotype combinations. Average age at death, however, was later for $B^{13}B^{13}$ than for $B^{21}B^{21}$ or heterozygous chicks (Table 1). Sire and dam effects, interactions between them, and interactions of sire and dam with other main effects were not significant. Incidence of neural lesions was much greater than for visceral lesions (Table 2), but did not differ among sexes or haplotypes.

Discussion

The greater susceptibility of females than males to Marek's disease was consistent with the majority of the literature (e.g. Crittenden et al., 1972; Zeitlin et al., 1972), although contrasting with previous observations for line LA (Dunnington et al., 1986). The expected greater resistance of $B^{21}B^{21}$ chicks was not evident in this experiment where a natural exposure to Marek's disease was used. Studies using several different challenge strains of Marek's disease virus produced varying levels of mortality which sometimes masked genetic differences in resistance (Schat et al., 1981; Calnek, 1985). In comparisons of 4 lines of chickens differing at the MHC, genetic differences occuring in response to the JM-10 strain of virus were suppressed when GA-5 or RB-1B strains were used (Schat et al., 1981). This masking effect may have contributed to the similarity among *B* haplotypes in this experiment.

Most previous reports of B^{13} susceptibility have been for heterozygotes (Briles et al., 1976; 1980). MHC-related resistance in heterozygotes is affected by the particular combination of alleles involved and/or the background genome (Hartmann et al., 1986). In crosses of a line (G) segregating at the MHC with line M (B^{2} homozygous) or line R (B^{15} homozygous), differences in Marek's resistance between B^{13} heterozygotes and B^{21} heterozygotes were apparent in offspring of G x M but not G x R matings (Hartmann et al., 1986). Susceptibility of $B^{13}B^{13}$ homozygotes has also been reported. In a challenge involving $B^{13}B^{13}$, $B^{19}B^{19}$, and $B^{21}B^{21}$ lines, incidence of Marek's disease for $B^{13}B^{13}$, while

similar to $B^{19}B^{19}$, was higher than for $B^{21}B^{21}$ (Schat et al., 1981). Since these lines were derived from different background genomes, there was confounding of line with *B* haplotypes.

There is precedence for non-MHC related differences in susceptibility in RPRL lines 6 and 7, which are both B^2B^2 but differ in resistance to Marek's disease (Stone, 1969). Such genes may also have influenced susceptibility in this trial. Non-MHC linked genes affecting susceptibility to Marek's have been identified (Fredericksen et al., 1982) and have been implicated in differences between RPRL lines 6 and 7. Epistasis between the $Ly-4^b$ and $Th-1^a$ alleles was associated with resistance to Marek's disease in B^5 homozygous F_7 crosses of 6_3 x 15_1 lines (Fredericksen et al., 1982). Lack of differences in mortality between MHC haplotypes in the present and some previous experiments indicates that although specific haplotypes may enhance resistance to disease under many circumstances, other factors such as background genome, strain of virus, and environmental and management differences may act to prevent expression of such resistance.

Summary

In the 12th generation of selection for high (HA) and low (LA) antibody response to sheep erythrocytes, sublines for major histocompatibility complex (MHC) haplotypes B^{13} and B^{21} were established. Heterozygous matings within the line LA background genome were made to test for effects of MHC haplotypes on resistance to Marek's disease. Chicks were not vaccinated for Marek's disease at hatch and were placed in floor pens with used litter. Mortality from Marek's disease commenced at younger ages in females than males, and for $B^{13}B^{21}$ and $B^{21}B^{21}$ than $B^{13}B^{13}$ chicks. Cumulative mortality was high (56%) and was greater for females than males. There were no differences among B haplotypes for cumulative mortality due to Marek's disease.

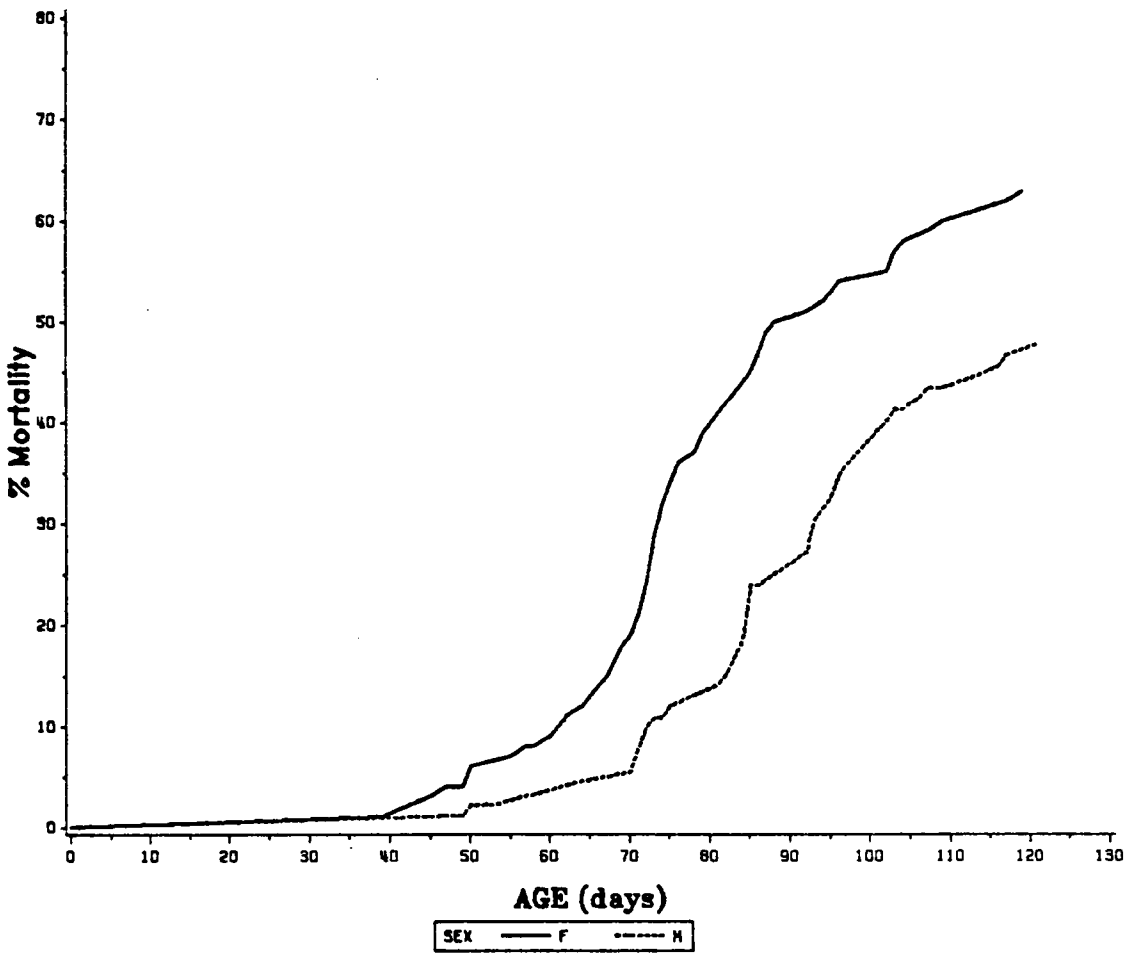

Figure 1. Cumulative mortality due to Marek's disease of male and female chicks from a line selected for low antibody response to sheep erythrocytes.

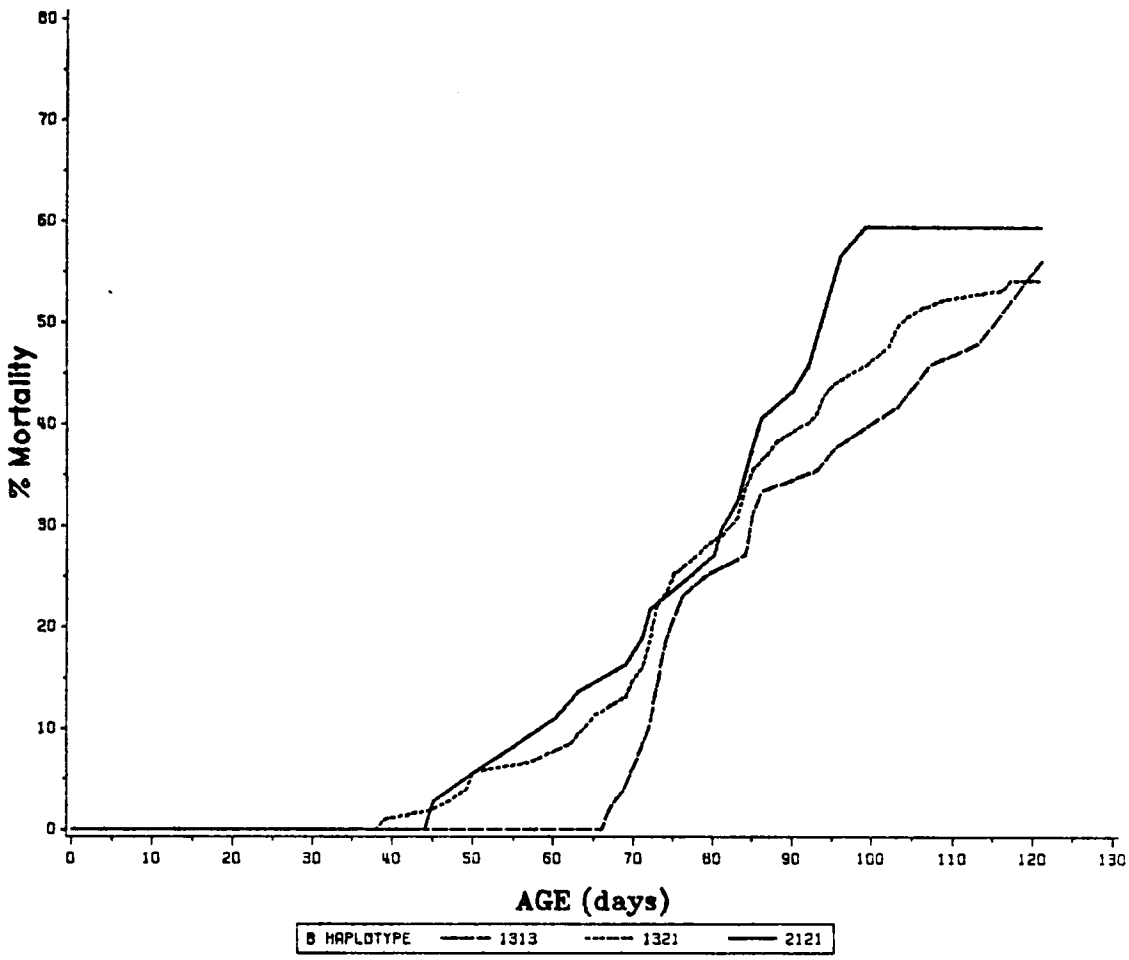

Figure 2. Cumulative mortality due to Marek's disease of chicks from a line selected for low antibody response to sheep erythrocytes and differing in *B* haplotype.

Table 1. Means and standard deviations of body weights and measurements of mortality due to Marek's disease of chickens selected for low antibody response to sheep red blood cells but differing at the *B* complex (MHC).

Haplotype	Body Weight (g) (g)	1st Death (d)	Age at Death (d)	Cumulative mortality (n)	(%)
$B^{13}B^{13}$	125 ± 31 a	67	89 ± 18 a	27/ 48 a	(56)
$B^{13}B^{21}$	130 ± 33 a	39	79 ± 18 b	58/107 a	(54)
$B^{21}B^{21}$	123 ± 34 a	45	78 ± 16 b	22/ 37 a	(59)
Sex					
M	140 ± 31 b	49	88 ± 16 a	44/ 92 a	(48)
F	118 ± 31 a	39	78 ± 18 b	63/100 b	(63)

[1] n = # dead from Marek's disease / # alive at onset.

a,b Means within a column having the same letter are not different, $p \geq .05$.

Table 2. Neural and visceral lesions due to Marek's disease in chickens selected for low antibody response to sheep red blood cells but differing at the *B* complex (MHC).

	Only Neural[1]		Only Visceral		Both Neural and Visceral	
Haplotype[2]	n	(%)	n	(%)	n	(%)
$B^{13}B^{13}$	16/ 48	(33)	2/ 48	(4)	9/ 48	(19)
$B^{13}B^{21}$	38/107	(36)	2/107	(2)	18/107	(17)
$B^{21}B^{21}$	15/ 37	(41)	1/ 37	(3)	6/ 37	(16)
Sex[2]						
M	28/ 92	(30)	1/ 92	(1)	15/ 92	(16)
F	41/100	(41)	4/100	(4)	18/100	(18)

[1] n = # having lesions / # alive at onset of Marek's disease.
[2] Comparisons of haplotypes and sexes made by chi-square test; no differences existed at $p \leq .05$.

CHAPTER III

SELECTION FOR HIGH OR LOW ANTIBODY RESPONSE TO SHEEP ERYTHROCYTES IN CHICKENS

Introduction

Genetic variation for antibody response to sheep erythrocytes has been demonstrated in several species including mice (Biozzi et al., 1970b), guinea pigs (Ibanez et al., 1980), and chickens (Siegel and Gross, 1980; van der Zijpp et al., 1983). In chickens, selection for high and low antibody production to sheep erythrocytes resulted in correlated responses in growth and reproductive traits (Siegel et al., 1982; Dunnington and Siegel, 1984; van der Zijpp et al., 1988), gene frequencies at alloantigen loci (Dunnington et al., 1984; van der Zijpp et al., 1987), and disease resistance (Gross et al., 1980; van der Zijpp et al., 1988). These correlated responses may affect fitness directly, as with changes in age at sexual maturity and egg production, or indirectly, as with modifications in disease resistance associated with specific haplotypes at the major histocompatibility complex (MHC), i.e., the *B* alloantigen system (Bacon, 1987). Presented here are comparisons of selected and unselected traits between and within lines of chickens selected for high or low antibody production to sheep erythrocytes.

Materials and Methods

Chickens used in these experiments were White Leghorn females from generations 10 through 14 of lines selected for high (HA) and low (LA) antibody response to a single intravenous injection of .1 ml of .25% suspension of sheep red blood cells (SRBC). Details of the selection procedures were described by Siegel and Gross (1980), and Siegel et al. (1982); only a brief description of husbandry and procedures are given here. Lines were reproduced in March of each year and chicks were reared as contemporaries in floor pens until 18 weeks of age, when they were individually caged. Antibody titers to SRBC were measured between 6 and 8 weeks of age in each generation. Through the third generation, SRBC antibody was measured by a tube dilution method, after which the method used was microtiter hemagglutination (Wegmann and Smithies, 1966). Plasma was obtained 5 days after injection with .1 ml of a .25% suspension of SRBC, and antibody titers were measured by microtiter hemagglutination method. Titers were expressed as $\log_2$ of the reciprocal of the highest dilution in which there was hemagglutination.

Genotypes at erythrocyte alloantigen systems *A, B, C, D, E, H, I* and *L* were determined by hemagglutination (Briles and Briles, 1982) between 2 and 4 weeks of age in generations 10 through 13. Gene frequencies presented in this paper were combined for males and females. Body weights were obtained at 4, 24 and 38 weeks of age, and on the day on which the first egg was laid (sexual maturity). Body weights at sexual maturity were not obtained in generation 10. Egg production was recorded daily

to 34 weeks of age in generation 10 and to 40 weeks of age in all subsequent generations. Normal and defective eggs were classified by the procedure of van Middelkoop and Siegel (1976). Mortality was recorded from 20 to 56 weeks of age.

Statistical analyses

Comparisons between lines were by analysis of variance, except for mortalities from 20 to 43 and 20 to 56 weeks of age, which were compared by Chi-square. Body weights and age at sexual maturity were transformed to logarithms prior to analysis. Hen-day ovulations (# ovulations / # days in egg production) and normal egg production (# normal eggs / # days in egg production), and percentages of normal, double-yolk, extra-calcified and compressed, broken, and other defective eggs were transformed to arc sines of the square roots. Heritabilities (h^2) of unselected traits and phenotypic and genetic correlations between them and antibody titers to SRBC were calculated from full-sib correlations (Becker, 1985). Negative variance components were set equal to zero for the calculations. Realized h^2 for SRBC titers were calculated for generations 10 through 14 as gain/reach in standard deviation units (Becker, 1985). Realized genetic correlations of antibody titers with other traits (Falconer, 1954) were calculated using realized h^2 for SRBC titers and h^2 based on full-sib correlations for all other values.

Results

Alloantigen systems

Gene frequencies for erythrocyte alloantigen systems *A, B, C, H,* and *L* were at or near fixation in one or both lines in generation 10 (Table 1). Changes were, however, observed in subsequent generations for gene frequencies not near fixation in generation 10. Between generations 10 and 11 frequency of C^4 doubled and C^2 decreased in line HA, and then remained stable. In line LA C^5 became fixed by generation 13. There was increasing divergence of lines HA and LA at the *B* and *D* loci, with increasing proportions of D^4 in line LA and of D^3 in line HA. Proportion of B^{13} in line LA remained stable, and B^{21} increased greatly in line HA, while B^{31} disappeared.

The unidentified allele at the *A* locus in generation 10 reported by Dunnington et al. (1984) was identified as a variant of A^2, and has been included in that category in all generations presented here. The *A* and *E* loci are linked (.5 centimorgans; Briles, 1968) and thus changes in gene frequency are not independent. The most common linkage group (haplotype) in both lines was A^4E^1 (Table 2). In line HA, A^2E^2 and A^1E^1 were at moderate frequency, and both increased in frequency to generation 13. Frequencies of *A-E* in line LA changed little between generations 10 and 13.

Comparisons between lines

The selected trait. Lines HA and LA continued to respond to selection for antibody titers to SRBC through generation 14 (Table 3), with major changes occuring in frequency distributions of titers (Figure 1). In generation 6, population means were distinctly different although ranges overlapped. Although bimodality was evident in generation 10 in both lines, by generation 14 much of it had disappeared and there was little overlap between lines. The difference between lines increased in later generations.

Unselected traits. Body weights were greater for line LA than HA pullets at 4 and 24 weeks of age, while the reverse was observed at 38 weeks (Table 4). Realized genetic correlations of the selected trait with body weights were negative and moderate to 24 weeks of age and positive and very low at 38 weeks (Table 4). Egg production commenced at lower body weights and at younger ages in line LA than HA, with the realized genetic correlations being .15 and .71, respectively.

Egg production was measured as a percentage of days between sexual maturity and a fixed age (hen-day egg production). Line LA had greater hen-day production of total ovulations and normal eggs in generation 14 but not in generation 10. The realized genetic correlation between SRBC titers and hen-day production was moderately low and negative. Adult mortality did not differ between lines.

Normal eggs as a percentage of total ovulations did not differ between lines. The percentage of double-yolk eggs was greater in line LA than HA in generations 13 and 14. There were no differences between lines

for percentage of extra-calcified, broken or other defective eggs when comparisons were made singly for each or when pooled. Realized genetic correlations of the selected trait with percentage of normal, double-yolk, and other defective eggs ranged from -.22 to .12 (Table 4).

Comparisons within lines

Heritabilities. Realized heritabilities of SRBC antibody titers for generations 10 through 14 were .23 in line LA and .25 in line HA. In both lines h^2 for body weights were moderate to low (Table 5), as were those for age at sexual maturity. Heritabilities for egg production traits were greater in line HA than LA, with those in line LA being near zero and those in line HA being moderately low.

Correlations. Regardless of line, phenotypic correlations of antibody titers with other traits were very small, ranging from -.12 to .09, while genetic correlations were consistently greater than phenotypic correlations (Table 5). In line HA genetic correlations for body weights were negative at all ages, as were phenotypic correlations (when not equal to zero). The smallest genetic correlation for body weight was that at sexual maturity. Genetic and phenotypic correlations for age at first egg were near zero in line HA. Genetic correlations for reproductive traits, other than age at first egg, were moderate with those for traits associated with production of normal eggs being positive, while those for percentage double-yolks and other defects were generally negative.

In line LA, genetic and phenotypic correlations for body weights were negative at 4 weeks of age and positive thereafter. Again the smallest

genetic correlation for body weight was that at sexual maturity. The genetic correlation for age at sexual maturity in line LA was negative and moderately low. Those for other reproductive traits were moderate to high, and followed the trend observed in line HA.

Discussion

Alloantigen systems

The increase in frequency of B^{21} in line HA in response to selection indicated a role for the MHC in antibody response to SRBC. In a similar selection experiment, van der Zijpp et al. (1987) also observed changes in gene frequency at the *B* complex, with increases in frequency of B^{21} in the line selected for high antibody response. The high proportion of B^{13} in line LA is not surprising because in the line LA background genome $B^{13}B^{13}$ chicks had lower titers to SRBC than $B^{21}B^{21}$ chicks (Dunnington et al., 1988). Haplotype B^{21} did, however, persist in the LA population, perhaps indicating a fitness role for that haplotype. In mice selected for high and low response to SRBC, differences in haplotypes at the MHC were observed (Colombani et al., 1979), with MHC haplotypes accounting for 12 to 20 % of the variability in antibody response to the selection dose (Mouton et al., 1979).

In chickens, some MHC haplotypes influence resistance to certain diseases (Bacon, 1987). Thus, changes in frequency of MHC haplotypes may be associated with differences between these lines in disease resistance reported by Gross et al. (1980). Changes in gene frequency at other allosystems may also be associated with variation in immunoresponsiveness. The *A-E* and *I* loci are associated with susceptibility to cecal coccidiosis (Johnson and Edgar, 1984; Martin et al., 1986), and the *C* locus is a minor histocompatibility locus (Schiermann and Nordskog, 1965).

The selected trait

The decreased mean response to SRBC in line LA may be attributed to increases in the proportion of nonresponders. This nonresponse to SRBC may have implications for overall immunoresponsiveness, although at higher or lower dosages of SRBC the frequency of nonresponders may be altered accordingly (Mouton et al., 1979; Ubosi et al., 1985b), implying a threshold. The lower mean for antibody titers in line HA in generation 10 appeared to be caused by an unusually high number of nonresponders in that generation, as well as bimodality.

Moderate realized heritabilities for these 5 generations were similar in magnitude to those obtained in the early generations for these lines (Siegel and Gross, 1980) and indicated continuing effectiveness of selection. In a similar selection experiment using chickens, realized heritabilities of .20 in the upward and .52 in the downward directions were obtained after 4 generations of selection (van der Zijpp et al., 1987).

Unselected traits

Negative correlated responses in body weight to selection for SRBC antibody were similar to those described by Siegel et al. (1982), and the persistence of such differences across generations suggested pleiotropy. Genetic correlations indicated that continued selection for antibody response will result in further divergence in juvenile body weight. These effects may be due to allocation in line HA of resources toward the immune

system rather than for growth. A differential allocation of resources is consistent with the observations in lines of chickens selected for high and low body weight at 8 weeks of age. A negative relationship existed between body weight and titers to SRBC, such that the low body weight line had the higher titers, and titers for the high body weight line were similar to those seen for line LA (Mauldin et al., 1978). Similarly, seven commercial chicken meat stocks (where the primary selection trait was juvenile body weight) had titers to SRBC similar to those observed in line LA (Siegel et al., 1984).

The direction of change and magnitude of genetic correlations between antibody response and juvenile body weight were similar to those observed in other lines of chickens selected for response to SRBC (van der Zijpp et al., 1987). Initial genetic correlation of 57-day body weight with antibody to SRBC was -.57, and after 4 generations of selection, higher 57-day body weights were observed in the line selected for low antibody response (van der Zijpp et al., 1987). Chicks selected for a high titer index to Newcastle disease virus and Escherichia coli, however, had heavier body weights at 7 weeks of age than those from the line selected for a low titer index (Pitcovski et al., 1987), indicating that correlations between juvenile body weight and antibody titers are specific for particular antigens or populations.

Earlier sexual maturity in line LA than HA may be due to attaining the necessary body weight for onset of lay at a younger age (Dunnington and Siegel, 1984). Differences between lines for age at sexual maturity were similar to those observed in earlier generations (Dunnington and

Siegel, 1984). Because no difference between lines for body weight at sexual maturity was previously found (Dunnington and Siegel, 1984), there may have been a change in the threshold body weight necessary for onset of egg production for these two lines in later generations.

The greater hen-day egg production by line LA than HA in some generations but not others was consistent with earlier reports for these lines (Siegel et al., 1982). The very low phenotypic correlations relative to the moderate to high genetic correlations indicated masking of genetic potential by the environment. Lack of differences between lines in adult livability, however, indicated that differential adult mortality was not a factor in such masking. The moderate, negative genetic correlations of the selected trait with hen-day ovulations and percentage normal eggs suggested that ova production changed somewhat during selection, tending to increase in line LA relative to line HA.

The persistence of correlated responses to selection for SRBC antibody is indicative of pleiotropic effects of genes associated with immunoresponse. Such effects are consistent with reports from other selection studies (van der Zijpp et al., 1987; Pitcovski et al., 1987), and with the decreased immunoresponsiveness of chickens selected for meat production (Siegel et al., 1982; 1984). The negative relationship between immunoresponsiveness and production traits suggests that natural selection favors an intermediate optimum in immune response, because overproduction of antibody has a negative selective value. Such overproduction usurps the animal's resources from other metabolic path-

ways, as indicated by negative genetic correlations with body weight and egg production.

Summary

Selection of chickens for high (HA) and for low (LA) antibody titers to sheep erythrocytes has resulted in differences in the selected trait and correlated responses in body weight, egg production and erythrocyte antigens. Response to selection continued through 14 generations. There was considerable divergence between lines for erythrocyte alloantigen systems, including the major histocompatibility complex. Females from line LA were heavier as juveniles, lighter as adults, matured at a younger age, and had higher egg production than those from line HA. There were no differences between lines for incidence of defective eggs laid, except for percentage of eggs with double-yolks, which was greater for line LA than HA. Phenotypic correlations of antibody response with growth and with reproductive traits were very low while genetic correlations were moderate to high for most of these traits.

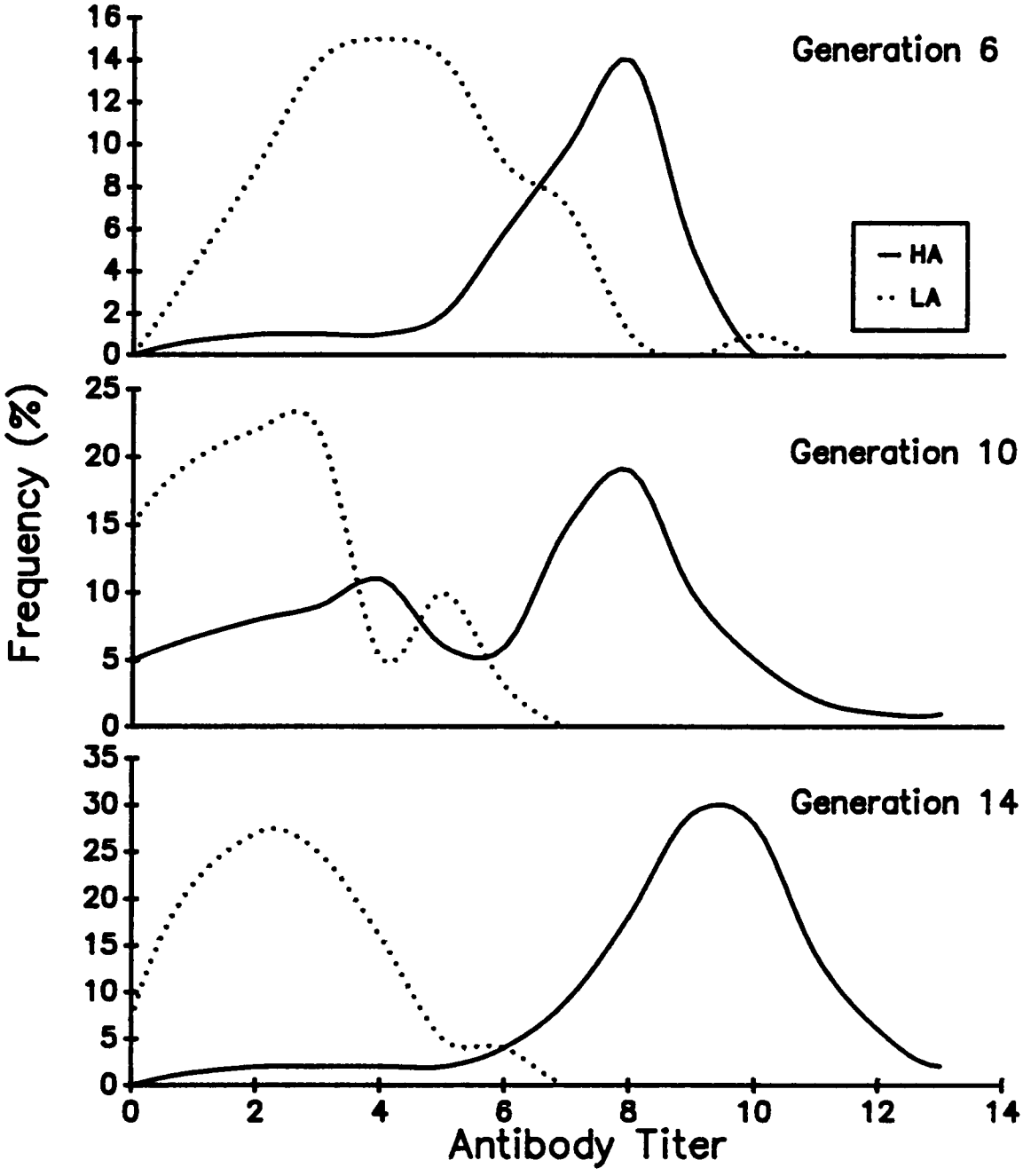

Figure 1. Frequency distributions of antibody titers to sheep erythrocytes for female chickens from lines selected for 6, 10, and 14 generations for high (HA) and low (LA) antibody response to sheep erythrocytes.

Table 1. Gene frequencies for alloantigen systems in lines of chickens selected for high (HA) and low (LA) antibody response to sheep erythrocytes in generations 10 and 13 of selection.

	Gene Frequencies[1]						
Allele	*A*	*B*	*C*	*D*	*E*	*H*	*I*
Generation 10 (modified from Dunnington et al., 1984)							
1	16/ 0			29/51	73/92	0/26	
2	24/ 2		40/ 5		27/ 8	100/74	32/33
3				71/39			
4	60/98		9/ 0	0/10			68/67
5			51/95				
13		15/99					
21		80/ 1					
31		5/ 0					
Generation 13							
1	24/ 0			9/37	69/95	0/25	
2	31/ 2		28/ 0		31/ 5	100/75	24/37
3				91/32			
4	45/98		19/ 0	0/31			76/63
5			53/100				
13		1/98					
21		99/ 2					
31		0/ 0					

[1] Gene frequencies (%) for lines HA and LA are indicated to the left and right of the slash (/), respectively. Both lines were L^2L^2.

Table 2. Frequencies of *A-E* allelic combinations (haplotypes) for lines of chickens selected for high (HA) and low (LA) antibody response to sheep erythrocytes.

A-E Haplotypes	Gene Frequencies[1] in Generation 10	13
1 1	16/ 0	23/ 0
2 1	2/ 0	1/ 0
2 2	22/ 1	31/ 2
4 1	55/92	45/95
4 2	5/ 7	0/ 3

[1] Haplotype frequencies (%) for lines HA and LA are indicated to the left and right of the slash (/), respectively.

Table 3. Mean antibody titers of females from lines of chickens selected for 14 generations for high (HA) and low (LA) antibody response to sheep erythrocytes and differences between the lines (HA - LA).

	Antibody titer[1]		
Generation	HA	LA	HA - LA
0	6.6	6.6	0
6	7.0	4.4	2.6
10	6.0	2.6	3.4
11	8.0	5.0	3.0
12	3.4	1.2	2.2
13	9.8	1.5	8.3
14	8.4	2.5	5.9

[1] In the first 3 generations of selection the antibody titers were measured by a tube dilution method. The correlation between that and the current microtiter method was .70 (Siegel et al., 1982).

Table 4. Means of unselected traits for females from lines of chickens selected for 10 and 14 generations for high (HA) and low (LA) antibody response to sheep erythrocytes.

Trait	Generation 10			Generation 14			Realized r GG
	HA		LA	HA		LA	
Body weight (g) at							
4 wk	203	**	224	149	**	185	-.64
24 wk	1287	**	1337	1270	**	1381	-.25
Sexual maturity[1]	1492	**	1431	1428	**	1376	.15
38 wk	1614	**	1505	1586	**	1557	.08
Reproductive traits							
Age at sexual maturity	183	**	170	186	**	164	.71
% Hen-day production of							
ovulations	71		74	74	**	80	-.28
normal eggs	69		72	73	**	78	-.22
% Of eggs that were							
normal[2]	97.2		97.8	98.4		98.6	-.04
double-yolk	.6		.7	.3	**	.6	-.20
other defects	1.0		1.5	1.3		.8	.12
Mortality[3]							
20 to 43 wks	4/105		9/100	2/90		5/90	
20 to 56 wks	16/105		17/100	3/90		5/90	

** Means within a row subheading are significantly different ($p \leq .05$).
[1] Not measured in S-10; values are for S-11.
[2] % Normal = # normal eggs / total # eggs laid.
[3] n = # dead at 43 or 56 weeks of age / # alive at 20 weeks of age.

Table 5. Heritabilities, genetic and phenotypic correlations from full-sib correlations between production traits and antibody titers in lines of chickens selected for high (HA) and low (LA) antibody response to sheep erythrocytes (generations 10 - 14).

Trait	HA h^2	LA h^2	HA r_{GG}	HA r_{PP}	LA r_{GG}	LA r_{PP}
Body weight at						
4 wk	.08	.09	-.42	-.11	-1.01	-.12
24 wk	.18	.17	-.17	.00	.16	.06
Sexual maturity[1]	.16	.17	-.09	-.05	.09	.05
38 wk	.20	.08	-.17	.00	.41	.09
Reproductive traits						
Age at sexual maturity	.16	.12	.00	-.03	-.28	.02
% Hen-day production of						
ovulations	.14	.03	.21	.02	.49	.03
normal eggs	.15	.02	.28	.03	.87	-.01
% Of eggs that were						
normal[2]	.12	.03	.43	.04	.68	-.08
double-yolk	.07	.04	-.50	.07	-.63	.01
other defects	.10	.02	-.34	-.02	-.48	.06

[1] For body weight at sexual maturity, values are for generations 11-14.
[2] % Normal = # normal eggs / total # eggs laid.

CHAPTER IV

THIOURACIL AND ANTIBODY TITERS OF CHICKENS FROM LINES SELECTED FOR HIGH OR LOW ANTIBODY RESPONSE TO SHEEP ERYTHROCYTES

Introduction

Although immune responses of thyroidectomized rats are lower than those of normal rats (Fabris, 1973), in chickens the results from experiments in which thyroid hormones were reduced chemically with goitrogens (Yam et al., 1981; Mashaly et al., 1983; Scott et al., 1985), genetically through use of the sex-linked dwarfing allele (Marsh, 1983; Marsh et al., 1984a; 1984b) or surgically (Mashaly et al., 1983) have been variable. Supplementation of thyroid hormones to normal chickens has had no effect on immunoresponsiveness, and results with thyroid-depleted chickens have been mixed (Yam et al., 1981; Marsh et al., 1984a; 1984b).

Factors such as genetic stock, age, and dosages used may have interacting effects with thyroid hormone levels on immune responses (Marsh, 1983; Chapter V). The experiment presented here was undertaken to examine the effects of reduction of thyroid hormone levels of chickens selected from a common background for high and low antibody response, using procedures and dosages under which the chickens had been selected.

Materials and Methods

Lines of White Leghorns differing in antibody response were developed from the Cornell Randombred population through divergent selection for antibody titers 5 days after an intravenous injection of .1 ml of a .25% suspension of sheep red blood cells (Siegel and Gross, 1980). At 1 day of age chicks from 13th generation matings of these high (HA) and low (LA) antibody producing lines were vaccinated against Marek's disease and randomized into wire brooder cages with 10 chicks per cage. Two diets were fed; one was a corn-soy diet containing 20% protein and 2685 Kcal/kg energy, and the other was the same diet with .1% thiouracil added. Feed and water were available ad libitum throughout the experiment and artificial illumination was continuous. All injections and bleedings in the experiment were done between 0800 and 0900 hours to avoid diurnal effects on T_3 and T_4 levels (Klandorf and Sharp, 1985).

Trial 1

All chicks in this trial were males, with 40 per line fed thiouracil and 20 per line fed the control diet. Individual body weights and feed intake per pen were obtained at 7, 14, 21, 28, 37, and 61 days of age. At 37 days of age all chicks were injected intravenously with .1 ml of a .25% suspension of sheep red blood cells (SRBC). Because previous experiments using these procedures showed peak titers 5 days after inoculation (Ubosi et al., 1985b), blood was obtained from the brachial vein 5 days post-primary inoculation (PPI) for determination of plasma

thyroxine (T_4) and tri-iodothyronine (T_3) concentrations and antibody levels to SRBC. Antibody response was determined by the microtiter hemagglutination method (Wegmann and Smithies, 1966), and titers were expressed as $\log_2$ of the reciprocal of the highest dilution showing hemagglutination. Plasma concentrations of T_3 and T_4 were determined by a double antibody radioimmunoassay verified for use on avian plasma (McNabb and Hughes, 1983). At 61 days of age a random sample of half of the birds in each line-diet subclass were given a booster dose (.1 ml of .25% suspension) of SRBC. All chicks were bled 3 days post-secondary inoculation (PSI) to determine antibody levels to SRBC. Previous experiments using these procedures showed peak antibody titers at that time (Ubosi et al., 1985b).

Trial 2

This trial involved males and females, with approximately 40 chicks per line-diet subclass. Individual body weights were obtained at 7, 14, 21, 28, 38 and 49 days of age. Twenty chicks per line-diet subclass were injected with SRBC at 21 days of age and the remainder were injected at 38 days of age, using the same dosage as in Trial 1. After each inoculation, 10 chicks per line-diet subclass were bled 3 and 7 days PPI and 10 were bled 5 and 10 days PPI. Total titers to SRBC were measured as in Trial 1, and 2-mercaptoethanol resistant (MER) titers to SRBC were determined as a measure of IgG (Delhanty and Solomon, 1966). In addition, 10 chicks per line-diet subclass were bled at 21 or at 48 days of age to obtain heterophil and lymphocyte counts. Blood smears were stained with

May-Grunwald and Geimsa stains, and cells were counted to a total of 60. The ratio of heterophils to lymphocytes (H/L) is altered when chickens are stressed (Gross and Siegel, 1983).

Statistical analyses

Effects of line, diet and the interaction of line and diet on body weights, feed efficiency, SRBC titers, T_3, T_4 and T_3/T_4 levels, and H/L ratio were evaluated by two-way analysis of variance. Body weights were transformed to natural logarithms and titers to square roots prior to analysis. Where line x diet interactions were significant analyses of variance were conducted within line and within diet. Differences discussed in the text are significant at $p \leq .05$. Feed efficiency data for Trial 1 were analyzed on a pen basis and all other traits on an individual bird basis. Product-moment correlations of T_3 and T_4 with antibody titers to SRBC were calculated in Trial 1.

In Trial 2, analyses of variance were also conducted to determine if age or sex were significant sources of variation for SRBC titers or H/L ratios. Since there were no differences between sexes, nor were any of the interactions between sex and other main variables significant, sexes were pooled for analyses of these traits. Because titers 7 and 10 days PPI and H/L ratios differed with age, analyses for all titers and for H/L ratios were done within ages.

Results

Because there was no line by diet interaction for body weight or feed efficiency at any age, means for these traits are presented for main effects only (Tables 1 and 2). In Trial 2, which included both sexes, males were heavier than females after 7 days of age. Since interactions of sex with other main variables were not significant, means are presented for males only. After 14 days of age in Trial 1, and by 7 days of age in Trial 2, line LA chicks were consistently heavier than those from line HA. An exception was at 61 days of age in Trial 1. Thiouracil-fed (TF) chicks had lower body weights than controls by 14 days of age in both trials. Feed consumption was lower in TF than control chicks, and feed efficiencies were greater for line LA than HA and for TF than control chicks after 28 days of age (Table 2).

Concentrations of T_3 in TF chicks were lower in HA than LA chicks (Table 3). In contrast, for the control diet T_3 concentrations were lower in LA than in HA chicks. This crossover pattern resulted in a significant line x diet interaction not only for T_3 concentrations, but for T_3/T_4 ratios. There was no difference between lines for T_4, but differences in T_4 were found between diets, with no apparent interaction of line x diet. All TF chicks had T_4 values below the assay sensitivity limits, i.e., < 1.25 ng/ml, and ratios of T_3/T_4 were calculated using a value of 1.25 ng/ml T_4 for TF chicks; thus, the true T_3/T_4 values for TF chicks are undoubtedly higher. Using these estimates, T_3/T_4 values for TF chicks were higher than those for controls, indicating that production of T_3 from

T_4 was not greatly impaired. Correlations of T_3 and T_4 with antibody titers were not significant.

Thiouracil feeding had no effect on H/L. Ratios were larger at 21 than 48 days of age, and for HA than LA chicks. Means and standard deviations at 21 days of age were .51 ± .26 for line HA, and .31 ± .13 for line LA. Respective values at 48 days were .37 ± .12 and .25 ± .13.

In Trial 1, line HA chicks had higher SRBC titers than line LA chicks at 5 and 27 days PPI, as well as 3 days after the booster was given (Table 4). Thiouracil had no effect on SRBC titers at any time, but in line HA primary titers were more persistent in TF chicks than controls, having a lower percentage change over time (57% vs 78%).

In Trial 2, antibody titers were greater for HA than LA chicks at all bleedings except 3 days PPI (Table 4). Effects of thiouracil feeding were transient and did not reveal any obvious trends. Interactions between line and diet for SRBC titers were significant 5 days PPI at both ages. When analyzed within lines at 21 days of age, TF chicks had lower 5-day titers than control chicks in line HA (6.4 vs 8.0) but did not differ in line LA (4.8 vs 4.3 for TF and control chicks, respectively). When analyzed within lines at 38 days of age there were no differences in 5-day titers due to thiouracil feeding, but trends were similar to those observed at 21 days of age. When analyzed within diets, line HA chicks had higher titers than line LA chicks at both ages.

MER titers were consistently greater in line HA than LA chicks (Table 5). The significant line x diet interaction 7 days PPI at the older age was due to an increase in MER titers of TF over control chicks in line

LA (3.3 vs 1.6) but not in line HA (5.3 vs 5.7). Thiouracil feeding did not affect MER titers at any other time.

Discussion

Hypothyroidism was successfully induced by thiouracil feeding, as indicated by greatly reduced plasma thyroid hormone concentrations and body weights. Feed efficiency was greater for TF than control chicks, although body weight and feed consumption were lower. Thiouracil feeding in chickens is known to reduce plasma T_3 and T_4 concentrations (Yam et al., 1981; Mashaly et al., 1983; Scott et al., 1985), body weights, and feed consumption (Yam et al., 1981; Mashaly et al., 1983; Scott et al., 1985; Glazener and Jull, 1946; Andrews and Schnetzler, 1946). Our observations of improved feed efficiency are consistent with some literature reports (Glazener and Jull, 1946) but not others (Andrews and Schnetzler, 1946), due perhaps to differences in age at initiation of thiouracil feeding (Kempster and Turner, 1945). Line differences in body weights (Ubosi et al., 1985a) and feed efficiency (unpublished) are consistent with previous experiments with these chickens, as are differences in total (Siegel and Gross, 1980; Gross et al., 1980; Ubosi et al.,1985a; 1985b) and MER (Ubosi et al., 1985b) titers to SRBC.

Literature reports of the relationship between antibody response to SRBC and reduction of thyroid hormones, through surgical or chemical means, have been conflicting. A line of New Hampshire chickens selected for low bursa weight differed from its base population in plasma T_3 concentrations when fed thiouracil, but not in titers to SRBC (Scott et al., 1985). Thyroidectomized White Leghorn chicks had higher secondary antibody response to SRBC than controls, while TF chicks did not differ from

controls in primary or secondary antibody titers (Mashaly et al., 1983). TF chicks did, however, have lower primary antibody titers to Brucella abortus, a thymus-independent antigen, than did controls or thyroidectomized chicks. In another experiment (Yam et al., 1981) thiouracil-fed White Leghorn chicks had significantly lower titers to SRBC than controls or chicks fed T_4 or thiouracil plus T_4.

Effects of supplemental thyroid hormones on growth and immune responses differ with initial thyroidal state of the fowl. Feeding T_4 to thiouracil-fed chickens prevented reductions of plasma thyroid hormone concentrations, growth, and antibody response (Yam et al., 1981; Scanes et al., 1984), but when fed to normal chickens supplementary T_4 or low levels of T_3 did not increase these traits (Marsh et al., 1984a; 1984b; May, 1980).

Conflicting reports in the literature concerning the effects of hypothyroidism on antibody response to SRBC may result from analyzing titers at different points on the antibody response curve. We found no differences in peak titers between TF and control chicks, but there appeared to be greater persistence of elevated titers in TF chicks in both trials. This trend was similar to that observed in lines of New Hampshire chicks fed thiouracil (Scott et al., 1985). In that experiment TF chicks did not differ from controls in antibody titers 4 or 5 days PPI, but 6 though 10 days PPI female TF chicks had higher antibody titers and greater persistence of titers than control chicks (Scott et al., 1985). Unlike our results, however, secondary antibody titers were lower for TF than control males and did not differ for females (Scott et al., 1985). We

found no differences in peak antibody response to a booster challenge and no differences in the reaction of males and females either PPI or PSI.

Inconsistent results from thiouracil feeding experiments regarding antibody response to SRBC demonstrate the importance of the different stocks used. Different dosages of SRBC antigen may also have affected the outcome of previous investigations since our results support that very high or very low dosages may mask genetic differences in antibody response (van der Zijpp, 1983b; Ubosi et al., 1985b). The dosage and procedures used in the present study were those under which the lines had been selected, and numerous reports using these procedures confirm consistent differences in antibody to SRBC by these lines (Siegel and Gross, 1980; Gross et al., 1980; Ubosi et al., 1985a; 1985b)

While line HA chicks had both higher T_3 levels and higher SRBC titers than those from line LA, the magnitude of difference between lines for T_3 concentrations was much smaller than the difference between TF and control chicks, which showed no accompanied difference in antibody titers. Correlations between T_3 concentrations and antibody titers were not significant, implying that an association between thyroid hormone levels and antibody titers was not a great factor in differences between selected lines. Since T_3 is involved in metabolic and growth processes in chickens (Scanes et al., 1984), and lines HA and LA differ in body weights, the differences in T_3 levels may partially account for body weight differences between lines.

It was thought that reduction in titers in other stocks fed thiouracil may have been due to stress caused by reduced feed consumption

of TF birds since protein-calorie malnutrition can reduce immunoresponsiveness (Good et al., 1980). Protein or caloric restriction of chickens reduced primary antibody titers to a high (5%) but not a low (.5%) dose of SRBC (Glick et al., 1981) indicating an interaction between level of nutrition and dosage in antibody response. Because H/L ratios are a reliable measure in chickens of response to stressors such as fasting, disease challenge, and social group instability (Gross and Siegel, 1983; Gross et al., 1984; Gross and Siegel, 1986), such data were obtained in Trial 2. While line and age differences were evident, there was no effect of thiouracil feeding on H/L, indicating that although TF chicks ate less and had lower body weights than controls this situation did not influence H/L ratios.

Many factors, including dosage of antigen (Marsh, 1983; van der Zijpp, 1983b; Ubosi et al., 1985b), route of administration (van der Zijpp et al., 1986), day when titers are measured (van der Zijpp, 1983b; Ubosi et al., 1985b) and vaccination history (van der Zijpp et al., 1982), can interact with genetic differences in antibody response. Data reported here do not show a reduction in plasma thyroid hormone concentrations to be one of these factors. In lines genetically selected for divergence in SRBC antibody production, genetic differences in antibody response are not overcome by reduction in thyroid hormone levels. This suggests a relatively minor role of T_3 and T_4 in modifying antibody response to sheep erythrocytes.

Summary

Chickens from lines selected for high (HA) or low (LA) antibody response to sheep red blood cells (SRBC) were fed ad libitum either a diet containing 0 (control) or .1% thiouracil (TF) throughout two trials. Chicks were injected intravenously with .1 ml of .25% SRBC at 37 days of age in Trial 1, and a booster of the same dosage was given to half of these chicks at 61 days of age. Antibody titers were measured 5 and 3 days after primary and secondary inoculations. In Trial 2, primary inoculations of SRBC were given at 21 and 38 days of age, and chicks were bled 3, 5, 7 and 10 days after inoculation. TF chicks had lower body weights and higher feed efficiencies than controls. Plasma thyroxine (T_4) concentrations were similar for both lines, but line x diet interactions were present for plasma tri-iodothyronine (T_3) and T_3/T_4 ratios. When fed the control diet, T_3 concentrations and T_3/T_4 ratios were higher for line HA than line LA, but the pattern was reversed when thiouracil was fed. Antibody titers to SRBC in both trials were higher for HA than LA chicks, but were similar for TF and control chicks. Persistence of elevated titers appeared to be greater in TF than control chicks. There were no differences between TF and control chicks for heterophil/lymphocyte ratios.

Table 1. Means and standard deviations of body weights (g) in Trials 1 and 2 for thiouracil (TF) and control (C) fed chickens of lines selected for high (HA) and low (LA) antibody response to sheep red blood cells.

	Line			Diet		
Age(d)	HA		LA	TF		C
			Trial 1			
7	46 ± 5		45 ± 5	45 ± 5		45 ± 4
14	69 ± 12		71 ± 11	65 ± 8	**	81 ± 9
21	102 ± 25	*	109 ± 26	91 ± 15	**	134 ± 16
28	148 ± 44	*	160 ± 46	126 ± 23	**	207 ± 26
37	214 ± 68	*	236 ± 72	181 ± 35	**	309 ± 39
61	441 ± 164		482 ± 146	373 ± 109	**	632 ± 65
			Trial 2[1]			
7	49 ± 5	**	56 ± 6	54 ± 8		52 ± 5
14	78 ± 11	**	94 ± 13	82 ± 13	**	92 ± 15
21	109 ± 16	**	139 ± 21	115 ± 20	**	136 ± 24
28	164 ± 27	**	197 ± 35	158 ± 25	**	201 ± 31
38	239 ± 42	**	276 ± 49	223 ± 34	**	287 ± 41
49	295 ± 61	**	338 ± 50	274 ± 47	**	351 ± 42

*, ** Adjacent means significantly different ($p \leq .05$; $p \leq .01$).
[1] Body weights in Trial 2 are presented for males only.

Table 2. Means and standard deviations of feed efficiencies (g body weight/g feed consumed)[1] in Trial 1 for thiouracil (TF) and control (C) fed chickens of lines selected for high (HA) and low (LA) antibody response to sheep red blood cells.

	Line			Diet		
Age (d)	HA		LA	TF		C
0-7	.24 ± .04		.26 ± .06	.26 ± .05		.22 ± .04
0-14	.28 ± .02		.32 ± .04	.33 ± .06		.35 ± .04
0-21	.33 ± .02		.36 ± .03	.42 ± .06		.40 ± .03
0-28	.34 ± .02		.38 ± .03	.41 ± .06		.39 ± .01
0-37	.33 ± .02	*	.37 ± .03	.36 ± .03	*	.29 ± .03
0-61	.31 ± .03	*	.33 ± .04	.32 ± .02	**	.25 ± .01

*, ** Adjacent means significantly different ($p \leq .05$; $p \leq .01$).
[1] Feed efficiency calculated on a per pen basis, n=6 pens per line n=8 TF and 4 control pens.

Table 3. Means and standard deviations of thyroid hormone levels in Trial 1 of thiouracil (TF) and control (C) fed chickens from lines selected for high (HA) and low (LA) antibody response to sheep red blood cells.

Trait[1]	Line	Diet		
		TF		C
T_3	HA	.545 ± .429	*	2.887 ± .451
		*		*
	LA	.724 ± .358	*	2.070 ± .414
T_4[2]	HA	< 1.25	**	12.730 ± 3.246
	LA	< 1.25	**	13.658 ± 3.225
T_3/T_4	HA	.429 ± .316	*	.251 ± .116
		*		*
	LA	.579 ± .287	*	.165 ± .075

*, ** Adjacent means significantly different ($p \leq .05$; $p \leq .01$).

[1] T_3 and T_4 concentrations expressed as ng/ml plasma.

[2] T_4 concentrations for TF chicks were below the sensitivity limits of the assay (< 1.25 ng / ml).

Table 4. Means and standard deviations of antibody titers in Trials 1 and 2 following primary and secondary inoculations with sheep red blood cells (SRBC) to lines selected for high (HA) and low (LA) antibody response to SRBC.

Age[1]	Days PPI	Line			Diet		
		HA		LA	TF		C
				Trial 1			
38	5	8.8 ± 3.5	**	4.2 ± 2.3	6.4 ± 3.5		6.8 ± 4.3
	27	3.4 ± 2.2	**	1.4 ± 1.5	2.7 ± 2.2		1.8 ± 1.9
	3 (PSI)	6.1 ± 2.3	**	3.3 ± 1.7	4.5 ± 2.4		5.0 ± 2.5
				Trial 2			
21	3	2.5 ± 1.0		2.8 ± 1.3	2.5 ± 1.3		2.8 ± 1.0
	5[2]	7.4 ± 1.4	**	4.6 ± 1.2	5.3 ± 1.3		6.1 ± 2.2
	7	7.9 ± 1.4	**	4.6 ± 1.3	6.2 ± 2.0		5.7 ± 2.2
	10	5.8 ± 0.9	**	4.0 ± 1.2	5.3 ± 1.2	**	4.2 ± 1.4
38	3	2.6 ± 0.7		2.4 ± 0.8	2.7 ± 0.7		2.2 ± 0.8
	5[2]	9.5 ± 2.1	**	4.2 ± 1.5	6.3 ± 2.7		6.4 ± 3.6
	7	11.7 ± 1.2	**	6.2 ± 1.9	9.1 ± 2.9	*	7.6 ± 3.4
	10	9.2 ± 2.3	**	5.3 ± 1.8	7.1 ± 2.4		6.6 ± 3.1

*, ** Adjacent means significantly different ($p \leq .05$; $p \leq .01$).
[1] Age at which antigen was administered.
[2] Significant line x diet interaction existed 5 days PPI.

Table 5. Means and standard deviations of 2-mercaptoethanol resistant (MER) antibody titers in Trial 2 following inoculation with sheep red blood cells (SRBC) to lines selected for high (HA) and low (LA) antibody response to SRBC.

Age[1]	Days PPI	Line			Diet	
		HA		LA	TF	C
21	3	0.8 ± 1.0	*	0.2 ± 0.4	0.5 ± 0.8	0.4 ± 0.7
	5	3.2 ± 1.4	**	0.3 ± 0.6	1.2 ± 1.5	1.8 ± 2.0
	7	3.4 ± 2.1	**	0.7 ± 0.9	1.4 ± 1.4	2.1 ± 2.4
	10	2.8 ± 1.7	*	0.4 ± 0.7	1.2 ± 1.6	1.4 ± 1.8
38	3	1.7 ± 0.6	*	0.1 ± 0.3	0.6 ± 0.9	0.8 ± 0.9
	5	6.2 ± 1.9	**	1.7 ± 1.1	3.3 ± 2.3	3.8 ± 3.0
	7[2]	5.5 ± 1.3	**	2.4 ± 1.5	4.1 ± 1.5	3.1 ± 2.4
	10	3.7 ± 1.4	**	1.5 ± 1.1	2.5 ± 1.4	2.4 ± 1.8

*, ** Adjacent means significantly different ($p \leq .05$; $p \leq .01$).
[1] Age at which antigen was administered.
[2] Significant line x genotype interaction existed at 7 days PPI.

CHAPTER V

THYROID HORMONES AND ANTIBODY RESPONSE TO SHEEP ERYTHROCYTES OF DWARF AND NORMAL CHICKENS SELECTED FOR JUVENILE BODY WEIGHT

Introduction

The effect of the sex-linked allele *dw* on immunoresponsiveness of chickens to sheep erythrocyte antigen (SRBC) appears variable (Marsh, 1983; Lilburn et al., 1986b). Although lower antibody levels were observed for dwarf than nondwarf White Leghorns (Marsh, 1983), there were no differences in meat-type chickens (Lilburn et al., 1986b). This inconsistency implies that effects of the dwarfing allele on responses to SRBC may be, as with other traits, dependent upon the background genome (Mauldin et al., 1978; Merat, 1982; Lilburn et al., 1986a).

Lower antibody titers to SRBC in sex-linked dwarf than in autosomal dwarf or normal Leghorns (Marsh, 1983) have been attributed to differences in serum concentrations of thyroid hormones (Marsh, 1983; Glick, 1984). This reasoning was consistent with findings that sex-linked dwarfs were functionally hypothyroid due to lower conversion rates of T_4 to T_3 (May and Marks, 1983; Scanes et al., 1983; Marsh et al., 1984a); T_3 is thought to be the metabolically active hormone in birds (see review in McNabb, 1987).

The following trials were conducted to further explore the influence of *dw* on antibody response to SRBC in lines of chickens selected for high and low juvenile body weight, and to evaluate the possible relationship with plasma thyroid hormone concentrations.

Materials and Methods

Stocks and husbandry

Chickens from 6 populations, HWN, LWN, HWD, LWD, HA and LA were used in this experiment. HWN and LWN were lines of nondwarf White Plymouth Rocks which had undergone individual phenotypic selection for high (HW) and for low (LW) body weight at 56 days of age (Siegel, 1962; Dunnington and Siegel, 1985). In the S_{13} generation the sex-linked dwarfing allele (*dw*) was introduced into these lines by mating Dw^{+}/dw males of a meat-type stock to a random sample of females from each line (Reddy et al., 1975). High weight dwarf (HWD) and low weight dwarf (LWD) populations were then developed through repeated backcrossing of Dw^{+}/dw males to normal HW and LW females. After 10 generations of backcrossing, the HWD and LWD populations were reproduced each generation by random mating of dwarf males and females within each population as contemporaries of the selected lines.

Lines HA and LA served as reference populations. These nondwarf lines of White Leghorns had undergone 14 generations of selection for high (HA) and low (LA) antibody response 5 days post intravenous (i.v.) inoculation with 0.1 ml of 0.25% suspension of sheep erythrocytes (Siegel and Gross, 1980). In the following trials antigen was inoculated i.v. into the brachial vein and blood samples were taken from this vein. Lines HA and LA do not differ in thyroxine levels at 42 days of age, but triiodothyronine levels are higher for line HA than LA (Chapter IV).

Trial 1

Chicks used in this trial were a random sample of males from generation 29 of populations HWN and LWN and generation 16 of the HWD and LWD sublines. All chicks were vaccinated at hatch against Marek's disease and reared as contemporaries in floor pens. Food, water and light were provided constantly throughout the experiment.

The test immunogen used in these trials was sheep red blood cells (SRBC), a T-cell dependent antigen in chickens. At 66 days of age 0.1 ml of either 0.025, 0.25, or 25% suspension of SRBC in physiological saline was injected i.v. into HWN, LWN, HWD and LWD chicks. There were 12 chicks per line-dosage subclass. Blood samples were collected from the brachial vein 5 and 12 days post primary inoculation (PPI). Experiments on the kinetics of response using these procedures showed that peak titers occurred 5 days PPI (Ubosi et al., 1985b). Plasma antibody to SRBC was determined by microtitration method (Wegmann and Smithies, 1966), and titers are expressed as $\log_2$ of the reciprocal of the highest dilution in which there was agglutination.

Secondary i.v. inoculations were given 24 days after primary inoculation to half of the chicks from each of the above populations. The booster dosage was 0.1 ml of 0.25% suspension of SRBC and chicks were bled 3 days post secondary inoculation (PSI). Previous studies (Ubosi et al., 1985b) with these procedures showed peak titers occurred at this time.

Twelve HA and 12 LA males served as a benchmark against which the titers of the above males could be compared. Each was inoculated i.v. with 0.1 ml of 0.25% SRBC at 52 days of age, (the same day as the primary

inoculation for the others), and were bled at the same time as the above males. Half were given the booster inoculation as well.

Trial 2

Chicks used in this trial were from generation 30 of the HWN and LWN populations and from generation 17 of the HWD and LWD populations. Male and female chicks from age-contemporary parents were vaccinated for Marek's disease at hatch and reared in wire brooder pens. As in Trial 1, feed and water were provided ad libitum and lighting was continuous.

Chicks were randomly divided into two groups at hatch. One group was inoculated i.v. with SRBC at 31 days of age, the other at 66 days of age. All chicks received 0.1 ml of 0.25% suspension of SRBC, the optimal dosage for discerning genetic differences in Trial 1. Blood was drawn 5 days PPI (at 36 and 71 days of age, respectively) for determination of SRBC antibody titers and plasma concentrations of triiodothyronine (T_3) and thyroxine (T_4). Antibody titers were assayed as in Trial 1. Concentrations of T_3 and T_4 were determined by double antibody radioimmunoassay procedures verified for use on avian plasma (McNabb and Hughes, 1983). Respective sample sizes of inoculated HWN, HWD, LWN, and LWD chicks were 31, 35, 27, and 21 at the younger, and 26, 35, 21, and 17 chicks at the older age.

Statistical analyses

Differences discussed in the text were considered statistically significant at $p \leq .05$. In Trial 1, analysis of variance was conducted

on antibody titers within days PPI or PSI to determine the effects of line, dosage, genotype, and interactions among these variables. The statistical model used was:

$$Y_{ijkl} = \mu + L_i + D_j + (LD)_{ij} + G_k + (LG)_{ik} + (DG)_{jk} + (LDG)_{ijk} + e_{ijkl}$$

where i = HW and LW lines, j = 1, 2, 3 dosages and k = Dw^+ and *dw* genotypes. Because means and variances were correlated for 5 day PPI titers at a 0.025% dose of SRBC, an analysis was conducted on these values transformed to natural logarithms. Overall results from this analysis did not differ from that for untransformed values. When dosage effects were significant, analyses of variance were conducted within dosage to determine line, genotype, and line x genotype effects.

For Trial 2, analysis of variance showed no sexual dimorphism for any trait measured. Therefore sexes were pooled and effects of age, line, genotype, and their interactions were determined by analysis of variance for antibody titers, plasma T_3 and T_4 concentrations, and T_3/T_4 ratios. Where age was significant, analyses were conducted within ages to determine effects of line, genotype, and line x genotype interaction.

Results

In Trial 1, means and standard deviations for body weight (g) at 56 days of age were 1501 ± 109 and 905 ± 97 for HWN and HWD chicks, respectively, and 247 ± 43 and 309 ± 54 for LWN and LWD chicks, respectively. The magnitude of these differences is typical for these lines as selection was for high and low 56-day body weight (Dunnington and Siegel, 1985). The heavier weights for LWD than for LWN chickens were due to continuing selection for low body weight for the latter and relaxed selection for the former. Mean 56-day body weights of HA and LA chicks, respectively, were 526 ± 100 and 651 ± 63, an expected result because of the negative correlated response between antibody titer to SRBC and body weight (Siegel et al., 1982; van der Zijpp et al., 1987).

SRBC titers

As expected, mean antibody titers for lines HA and LA using the 0.25% dosage of SRBC in Trial 1 were different both PPI and PSI, and bracketed those for the other populations 5 days PPI (Table 1). Thus, at this dose of antigen, responses of dwarf and normal HW and LW males were within the range of populations specifically selected for high and for low antibody response to SRBC antigen 5 days PPI.

Interactions of line and genotype with dosage were significant. The greatest separation among genotypes and lines was achieved using a 0.25% suspension of SRBC. Differences between genotypes were masked at the lower dose, while at the higher dose there was masking of all genetic differ-

ences. Primary and secondary SRBC titers were reduced at the lower dose of antigen and elevated at the higher dose. The exception was that the secondary response of LWD chicks did not differ with primary dosage level but was high for all dosages. Since differences between primary dosage groups existed for all SRBC titers, both PPI and PSI, results will be presented by dosage.

For the 0.025% dose of SRBC there were no line x genotype interactions for titers 5 and 12 days PPI, nor 3 days PSI (Table 1). Titers were consistently lower for HW than LW chicks. Although there was no effect of dwarf genotype on antibody titers at either 5 or 12 days PPI, titers for dwarfs were higher than those for normals 3 days PSI. At 27 days PPI, the line x genotype interaction was significant. The interaction resulted from dwarfs having higher titers than normals in line LW and no difference between genotypes in line HW (Table 2).

When chicks were injected with 0.25% SRBC, antibody titers 5 days PPI were higher for LW than HW, and dwarf than normal chicks, with no line x genotype interaction (Table 1). Twelve days PPI the interaction was significant, with dwarfs having higher titers than normals in line HW but not LW (Table 2). By 27 days PPI, titers were higher for LW than for HW chicks (Table 1), with no effect of alleles at the dwarf locus on residual titers. When given the booster, however, the background genome had no effect on secondary response while titers were higher for dwarf than nondwarf chicks.

For chicks inoculated with a suspension of 25% SRBC, there were no differences among genetic populations for titers 5 and 27 days PPI or 3

days PSI (Table 1). Twelve days PPI a line x genotype interaction existed with dwarfs having higher titers than normals in line HW but not line LW (Table 2). This pattern was due, in part, to a greater decrease in titers from 5 to 12 days for HWN chicks than for those from the other populations.

In Trial 2, titers at 36 and 71 days of age were different so analyses were conducted within ages. Interactions of line x genotype were not significant and hence marginal means are presented. At 36 days of age (5 days PPI) titers were higher for dwarf than normal chicks (30.1 ± 1.9 vs 2.2 ± 1.8), and for line LW than HW chicks (3.5 ± 1.8 vs 2.0 ± 1.7). At 71 days of age (5 days PPI) the pattern was similar, with titers of 6.7 ± 30.1 and 3.7 ± 2.9 for LW and HW chicks, respectively and 5.6 ± 3.0 for dwarfs and 4.3 ± 3.0 for normal chicks.

Thyroid hormones

Age effects (36 vs 71 days) and interactions of age with line and with genotype were significant for thyroid hormones and T_3/T_4 ratios. Accordingly, subsequent analyses were conducted within ages for these three traits. At the younger age there was no line x genotype interaction for plasma concentrations of T_3 (ng/ml), nor any difference between HW (1.38 ± .47) and LW (1.41 ± .54) chicks. Normal chicks had higher T_3 concentrations (1.65 ± .40) than dwarfs (1.02 ± .36). At 71 days of age, the interaction was significant because the difference between dwarfs and normals was significant in line LW but not in line HW (Table 3). Line x genotype interactions were significant for plasma T_4 concentrations at

both ages. At the younger age, T_4 was consistently higher for dwarf than normal chicks in line LW while there as no difference in line HW. In older chicks there was a crossover interaction with the higher values for normals in line HW and for dwarfs in line LW.

At 36 days of age there was no line x genotype interaction for T_3/T_4 ratios. There was no difference between lines; however, ratios were lower for dwarf than normal chicks. In older chicks there was a line x genotype interaction for T_3/T_4 ratios (Table 3). In line LW ratios were consistently greater for normal than dwarf chicks, while in line HW there was no difference between dwarf and normal chicks.

Discussion

Differences between lines in SRBC antibody response were dose dependent, with LW chicks having higher titers than HW chicks at the intermediate and lower doses. These line differences were also observed in previous experiments (Reddy et al., 1975; Mauldin et al., 1978; Marsteller et al., 1980). The lack of line differences at the higher dose of antigen was consistent with the masking of genetic differences in antibody response by using either high or low dosages of antigen (Ubosi et al., 1985b).

Within lines, dwarfs generally had higher peak SRBC titers than nondwarfs at the intermediate dose, while at other doses there were no differences between genotypes. Previous researchers reported similar results in line HW, but line LW dwarfs had lower titers than normals (Reddy et al., 1975; Mauldin et al., 1978; Marsteller et al., 1980). This change in line LW may be due to continued selection for low body weight in LWN but not in LWD in the intervening generations, which resulted in a shift where during early ages dwarfs are no longer smaller than normal chicks in line LW (Zelenka et al., 1986). Marsh (1983) showed that differences between dwarf and nondwarf Leghorns could be masked using high doses of SRBC.

The pattern of a difference in T_3 and T_4 concentrations at an older but not a younger age between HWN and LWN chicks is consistent with previous results for these populations (Nir et al., 1987). Because T_3 is considered to be the metabolically active thyroid hormone (see review by

McNabb, 1987), the lower plasma T_3 concentrations of HWN chicks at the older age may indicate a mild functionally hypothyroid condition in line HW. Plasma T_4 concentrations, however, were not decreased in HW chicks, suggesting that such hypothyroidism is a peripheral phenomenon, not one of thyroid gland origin.

Thyroid hormone concentrations of dwarf and normal chicks differed in lines HW and LW. Plasma T_4 of dwarf chicks was suggestive of "normal" activity in the thyroid gland since no reduction in T_4 was seen relative to nondwarf chicks. Differences between groups in plasma T_4, which is considered to be a prohormone, are probably less important than differences in T_3.

Lower plasma T_3 and T_3/T_4 ratios, and elevated T_4 concentrations of dwarfs relative to nondwarfs in line LW are suggestive of decreased peripheral deiodination in LW dwarfs. These results are consistent with those showing lower T_3 and higher T_4 concentrations in dwarf White Leghorn chickens (Scanes et al., 1983; Marsh et al., 1984a; 1984b) that resulted from decreased peripheral deiodination of T_4 to T_3 (Scanes et al., 1983). A similar pattern of hormone concentrations exists in meat-type stocks (May and Marks, 1983; Lauterio et al., 1986), although exceptions have been noted (Callahan and Parsons, 1986). In line HW there was less difference between dwarfs and normals for thyroid hormone concentrations, and reduced peripheral deiodination did not appear to be a factor at the older age. Thus, the expression of allele *dw* is influenced by the background genome, an observation noted for other traits (Mauldin et al., 1978; Merat, 1982; Lilburn et al., 1986a).

Previous studies using both Leghorns (Huybrechts et al., 1986) and meat-type chickens (Lilburn et al., 1986a) suggested that plasma T_3 and T_4 concentrations of dwarfs differ from normals only at younger ages, with the difference in T_3 concentrations persisting longer. Although we found no evidence of age-related patterns in plasma thyroid hormones in line LW, the picture for line HW was more complex and suggested age related changes. In line HW there was little change in plasma T_3 between 36 and 71 days of age in normal chicks, while in dwarfs the concomitant increase in plasma T_3 to 71 days of age overcame the earlier difference between genotypes.

A consistent relationship between plasma thyroid hormone concentrations and antibody response to SRBC was not apparent in these populations. Since previous researchers obtained results opposite from ours in antibody response to SRBC by dwarf chickens (Marsh, 1983), but plasma T_3 concentrations were similar to ours, plasma T_3 is probably not the causative factor in differences in antibody response to SRBC between dwarf and nondwarf chickens. Because the *dw* allele had similar effects on titers of both HW and LW chicks but had markedly different effects on plasma T_4 levels, T_4 probably does not directly affect immune response to SRBC. This conclusion also was reached by Marsh et al. (1984b) from experiments supplementing dwarfs with T_4. Although feed supplementation with T_3 or T_4 increased thymic weights and serum levels of T_3 and T_4 of both normal and dwarf chicks, there was no accompanying increase in antibody titers to SRBC (Marsh et al., 1984a; 1984b). While bursa weights of normal chicks fed T_3 or T_4 were increased, T_4 and T_3 supplementation

to dwarfs increased and decreased bursa weight, respectively (Marsh et al., 1984a; 1984b). The lack of a correlated response in SRBC titers is not surprising because the relationship of SRBC antibody levels with thymic and bursal weight has been equivocal (Yamamoto and Glick, 1982; Ubosi et al., 1985a).

Antibody responses to SRBC in trials where hypothyroidism was induced experimentally by feeding thiouracil have also been inconsistent. In some cases there were no differences between thiouracil-fed and control chicks (Mashaly et al., 1983; Chapter IV). In others, those fed thiouracil had higher (Scott et al., 1985) or lower (Yam et al., 1981) titers than controls. Thus, feeding experiments as well as those using populations known to differ in immunoresponsiveness and thyroid hormone concentrations suggested that a direct relationship between thyroid hormones and antibody response to SRBC is unlikely. This may be due to interactions of plasma thyroid hormone concentrations with factors such as age, dose and source of antigen, as well as the previous life history of the individuals and populations.

Summary

Experiments were conducted using lines of chickens selected for high (HW) and low (LW) body weight to determine effects of dosage of antigen, line, sex-linked dwarfism, and plasma thyroid hormone concentrations on antibody titers to sheep red blood cells (SRBC). The greatest separation of lines and of dwarf-normal genotypes occurred with an intermediate dose (0.1 ml of 0.25%) of SRBC. Five days after administration of SRBC at this dose antibody responses were greater for line LW than HW and in dwarf than normal chicks. The dwarf-normal but not the line relationship was evident for the booster challenge as well. Separation of lines and dwarf-normal genotypes for plasma thyroid hormone concentrations was greater at 71 than 36 days of age. Also the effect of the dwarfing allele on thyroid hormones was dependent on the background genome. There was no relationship between plasma triiodothyronine (T_3) and thyroxine (T_4) concentrations and antibody responses to SRBC. These data do not support the suggestions of a relationship between antibodies to SRBC and plasma T_3 and T_4.

Table 1. Means and standard deviations of antibody titers to sheep red blood cells for high (HW) and low (LW) body weight lines and for normal (N) and dwarf (dw) genotypes by antigen dose and days from primary (PPI) and secondary (PSI) inoculations.

Dose (%)	L	G	Days PPI 5	Days PPI 12	Days PPI 27	Days PSI 3
0.025	HW		2.4 ± 1.5	.6 ± .9	see Table 2 for Interactions	2.4 ± 1.5
			**	**		*
	LW		6.1 ± 2.9	2.3 ± 1.6		4.6 ± 2.7
		N	3.8 ± 2.4	1.3 ± 1.8		2.4 ± 1.2
			NS	NS		*
		dw	5.0 ± 3.4	1.7 ± 1.2		4.7 ± 2.9
0.25	HW		4.8 ± 2.7	see Table 2 for Interactions	1.2 ± .9	5.2 ± 1.3
			**		**	NS
	LW		10.3 ± 3.4		3.2 ± 2.0	5.8 ± 1.9
		N	6.0 ± 4.2		1.7 ± 1.8	4.8 ± 1.1
			**		NS	*
		dw	9.4 ± 3.3		2.8 ± 1.8	6.3 ± 1.9
	HA[1]		12.4 ± 1.8	6.2 ± 2.1	2.7 ± .8	5.8 ± 2.1
			**	**	*	*
	LA[1]		4.0 ± 2.2	1.6 ± 2.6	1.0 ± 1.1	3.2 ± 1.5
25	HW		13.2 ± 1.8	see Table 2 for Interactions	3.6 ± 2.3	6.1 ± 1.6
			NS		NS	NS
	LW		13.6 ± .5		4.8 ± 1.6	6.8 ± 1.7
		N	13.2 ± 1.6		4.1 ± 2.0	6.0 ± 1.8
			NS		NS	NS
		dw	13.6 ± .9		4.5 ± 2.1	7.0 ± 1.4

* $p \leq .05$; ** $p \leq .01$; NS not significant ($p \geq .05$).
L = line, G = genotype.
[1] Reference populations, selected for high (HA) or low (LA) antibody production to SRBC.
Boxes indicate significant line x genotype interactions (see Table 2).

Table 2. Means and standard deviations of antibody titers to sheep red blood cells for normal (N) and dwarf (dw) chickens *within* high (HW) and low (LW) body weight lines, by antigen dose and days from primary (PPI) and secondary (PSI) inoculation.

Dose (%)	L	G	Days PPI 5	Days PPI 12	Days PPI 27	Days PSI 3
0.025	HW	N	2.5 ± 1.7	.3 ± .6	.3 ± .8	2.0 ± 1.1
					NS	
		dw	2.3 ± 1.0	1.0 ± 1.1	.2 ± .5	2.8 ± 1.9
	LW	N	5.2 ± 2.3	2.2 ± 2.0	.3 ± .5	2.8 ± 1.3
					**	
		dw	7.0 ± 3.3	2.3 ± .9	2.0 ± 0.0	6.3 ± 2.7
0.25	HW	N	3.2 ± 2.0	2.1 ± 1.4	.9 ± 1.1	4.8 ± 1.2
				**		
		dw	6.9 ± 1.7	4.5 ± 1.1	1.6 ± .6	5.6 ± 1.5
	LW	N	9.2 ± 3.6	6.1 ± 3.8	2.7 ± 2.0	4.8 ± 1.2
				NS		
		dw	11.4 ± 2.8	5.3 ± 2.4	3.8 ± 2.0	6.8 ± 2.1
	HA[1]	N	12.4 ± 1.8	6.2 ± 2.1	2.7 ± .8	5.8 ± 2.1
	LA[1]	N	4.0 ± 2.2	1.6 ± 2.6	1.0 ± 1.1	3.2 ± 1.5
25	HW	N	12.8 ± 2.2	6.6 ± 2.0	3.3 ± 2.2	5.3 ± 1.8
				**		
		dw	13.6 ± 1.3	9.2 ± 1.7	4.0 ± 2.6	7.0 ± 1.0
	LW	N	13.7 ± .5	8.1 ± 1.9	4.8 ± 1.6	6.7 ± 1.8
				NS		
		dw	13.6 ± .5	8.1 ± 1.8	4.8 ± 1.7	7.0 ± 1.8

* $p \leq .05$; ** $p \leq .01$; NS not significant ($p \geq .05$).
L = line, G = genotype.
[1] Reference populations, selected for high (HA) or low (LA) antibody production to SRBC.
Boxes indicate significant line x genotype interactions. See Table 1 for line and genotype differences.

Table 3. Means and standard deviations of plasma concentrations (ng/ml) of T_3 and T_4 for normal (N) and dwarf (dw) genotypes within the high (HW) and low (LW) weight lines at 36 and 71 days of age.

Line	G	Age (days) 36	71
T_3			
HW	N	1.66 ± .33	1.74 ± .69
		**	NS
	dw	1.07 ± .39	1.49 ± .75
LW	N	1.63 ± .48	2.56 ± .96
		**	**
	dw	.86 ± .16	1.18 ± .62
T_4			
HW	N	8.79 ± 2.53	14.71 ± 3.97
		NS	*
	dw	8.95 ± 2.29	12.66 ± 3.45
LW	N	7.18 ± 2.75	11.62 ± 3.70
		**	**
	dw	10.58 ± 2.84	17.90 ± 2.76
T_3/T_4			
HW	N	.21 ± .10	.13 ± .06
		**	NS
	dw	.12 ± .04	.14 ± .10
LW	N	.29 ± .23	.25 ± .16
		**	**
	dw	.09 ± .02	.07 ± .03

* $p \leq .05$; ** $p \leq .01$; NS not significant ($p \geq .05$).
G = genotype.
Boxes indicate significant line x genotype interactions.

GENERAL SYNTHESIS

Costs of disease management in poultry through use of feed additives and prophylactic measures should be viewed not only in a monetary sense but also in terms of increased resistance of pathogens and decreased host genetic resistance. These factors have led to greater interest in improving disease resistance through breeding or gene transfer techniques. The experiments reported in this dissertation were designed to explore the interrelations of genetic variation in immunoresponsiveness with economically important traits, thyroid hormones, and other immunological measures. These experiments were conducted in both White Leghorn and White Plymouth Rock chickens.

Kinetics of 2-mercaptoethanol-resistant (MER) and sensitive (MES) antibodies to sheep erythrocytes differed between lines selected for high (HA) and low (LA) antibody response to sheep red blood cells (SRBC). MER and MES are measurements of IgG and IgM, respectively. Antibodies to SRBC were measured 3, 5, 7, 12, 27, and 29 days after primary and 3 and 5 days after secondary inoculation. Total antibody and persistence of response were greater in line HA than LA. Although levels of MES increased and then declined over time in line HA, they persisted at low levels in line LA. Titers of MER antibody were greater in line HA than LA, both on an absolute and relative basis. Secondary responses were similar to primary responses, however, in line HA the peak for MES occured before that for total titers. Thus, MER and MES responses were altered differentially according to the direction of selection. It is not known whether such

changes reflect differences in the number of antibody producing cells, or in the affinity of these cells for antigen. Immunological studies should be performed to address this issue.

A series of sublines at the major histocompatibility complex (MHC) was developed from lines HA and LA. These sublines allowed testing of the interaction of background genome and MHC haplotype for disease resistance. Combinations of MHC haplotypes included $B^{13}B^{13}$, $B^{13}B^{21}$, and $B^{21}B^{21}$ in both lines. An experiment using offspring of heterozygous matings in LA examined the role of these haplotypes in resistance to Marek's disease. Studies in other laboratories had shown $B^{21}B^{21}$ chickens to be highly resistant to Marek's disease. In this experiment, all haplotypes experienced high mortality (56%) to a natural exposure of Marek's disease, and there was no difference among haplotypes in cumulative mortality. The average age of death was later for $B^{13}B^{13}$ chicks, primarily because mortality in that group commenced at an older age. The lack of expected differences between MHC haplotypes suggests that background genome may influence responses observed from gene transfers. To test whether this is the case for resistance to Marek's disease, experiments are being conducted using these MHC haplotypes in both the HA and LA lines. Subsequent experiments could involve other infectious agents, and should compare the results for natural Marek's exposure with those from a controlled challenge.

Correlated responses to selection for SRBC antibody response were observed in females of lines HA and LA for alleles at erythrocyte alloantigen systems, body weight, and egg production traits. There were

considerable differences between lines for frequencies of alloalleles for erythrocyte antigens, including the MHC. Line LA females were heavier at younger ages, lighter as adults, matured at a younger age, and had greater egg production than those from line HA. For most traits, phenotypic correlations of antibody production with growth and reproductive traits were low, while genetic correlations were moderate. This situation for egg production may be indicative of lymphoid leukosis viremia, and this should be tested.

The negative genetic correlations of antibody response with production traits appear to fit the concept of resource allocation. This concept is consistent with that of genetic homeostasis (Lerner, 1954). As viewed in this dissertation, the resources of an animal must be allocated to competing needs such as maintenance, growth, reproduction, and response to the environment. An animal cannot maximize output to any one of these without taking away from others. Much of this allocation is determined genetically, while the rest will depend upon inputs from the environment, and the animal's perceptions of those inputs. Thus, selection for antibody response has genetically diverted resources from growth and reproduction for increased immunoresponsiveness.

Two experiments were conducted to explore the association of thyroid hormones with antibody response. In one experiment thiouracil was fed to lines known to differ in antibody response to SRBC. In the other, antibody response was measured in populations that differed in circulating thyroid hormone concentrations. When chickens from lines HA and LA were fed a control diet or one containing .1% thiouracil, those fed

thiouracil had lower plasma triiodothyronine (T_3) and thyroxine concentrations. When fed the control diet, T_3 concentrations were higher for line HA than LA, but the pattern was reversed when thiouracil was fed. Thyroxine concentrations were similar for both lines. There were no differences between lines for heterophil/lymphocyte ratios or antibody response to SRBC.

Plasma thyroid hormone concentrations and antibody responses to SRBC were measured in lines of White Plymouth Rock chickens selected for high and low 8-week body weight and in their respective dwarf sublines. Line x genotype interactions were present for thyroid hormone concentrations. In the low weight line, T_3 and T_4 were higher and lower, respectively, in normals than dwarfs. In the high weight line, at 36 days of age T_3 was higher in normals than dwarfs and T_4 concentrations did not differ, whereas at 71 days T_3 levels were similar and T_4 was higher in normals than dwarfs. An intermediate dose of SRBC was optimal for separating effects of line and dwarf-normal genotypes. Peak antibody responses were greater for the low than high weight line and for dwarf than normal chicks. Although secondary responses were greater for dwarf than normal chicks, lines responded similarly.

These experiments showed little if any relationship between thyroid hormone concentrations and antibody response to SRBC. They suggest that where such a relationship has been noted that a third factor such as stress or background genome was an influence. Differences in antibody response of dwarf and normal chickens show that single genes may have unexpected pleiotropic effects.

Selection for antibody response to a generalized antigen such as sheep erythrocytes can result in correlated responses not only in immunological parameters and disease resistance, but in other economic traits. The persistence of correlated responses in growth and reproductive traits indicates pleiotropy and, along with evidence of lower antibody production in broiler stocks, emphasizes the need for a balanced approach to selection in chickens. The lack of phenotypic correlations of these traits with antibody production implies that the correlated effects of selection may not be visible in the short term.

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APPENDIX

Appendix Table 1. Means ± standard deviations of body weight and adult mortality of female chickens of high (HA) and low (LA) antibody selected lines for generations 10 to 14, Chapter III.

Line	Generation 10	11	12	13	14
Body Weight (g)					
4 weeks					
HA	203 ± 32 **	176 ± 26 **	185 ± 39 **	159 ± 26 **	149 ± 31 **
LA	224 ± 32	198 ± 30	217 ± 31	195 ± 30	185 ± 23
24 weeks					
HA	1287 ± 152 **	1390 ± 139 **	1390 ± 152	1284 ± 99 **	1270 ± 181 **
LA	1337 ± 130	1423 ± 175	1447 ± 153	1363 ± 140	1381 ± 110
Sexual maturity					
HA	ND	1492 ± 140 **	1535 ± 152 **	1471 ± 128 **	1428 ± 130 **
LA	ND	1431 ± 148	1461 ± 121	1408 ± 121	1376 ± 107
38 weeks					
HA	1614 ± 185 **	1661 ± 168 **	1627 ± 198	1629 ± 138 **	1586 ± 152
LA	1505 ± 147	1605 ± 186	1587 ± 136	1579 ± 146	1557 ± 139
Mortality[1]					
20-43 weeks					
HA	4/105	3/ 86 **	11/78	1/90	2/90
LA	9/100	14/100	10/85	2/90	5/90
20-56 weeks					
HA	16/105	5/ 86	27/78	2/90	5/90
LA	17/100	21/100	20/85	2/90	5/90

*,** Lines are significantly different ($p \leq .05$; $p \leq .01$).
[1] n = # dead at 43 weeks / # alive at 20 weeks of age;

Appendix Table 2. Means ± standard deviations of reproductive traits of female chickens from high (HA) and low (LA) antibody selected lines for generations 10 to 14, Chapter III.

Line	Generation 10	11	12	13	14
Age at sexual maturity					
HA	183 ± 13	179 ± 12	187 ± 13	185 ± 10	186 ± 14
	**	**	**	**	**
LA	170 ± 11	162 ± 13	168 ± 8	169 ± 11	164 ± 9
% hen-day ovulations					
HA	70.9 ± 13.4	72.1 ± 13.9	63.3 ± 23.3	80.9 ± 8.3	74.0 ± 13.4
			**		**
LA	73.6 ± 10.5	71.2 ± 19.1	70.8 ± 22.5	80.4 ± 7.9	79.6 ± 6.8
% Hen-day normal eggs					
HA	69.2 ± 14.3	68.6 ± 14.9	60.1 ± 23.0	79.1 ± 10.1	72.8 ± 13.6
			**		**
LA	71.5 ± 10.5	68.3 ± 18.7	68.9 ± 22.1	78.1 ± 8.8	78.0 ± 7.2
% Normal eggs					
HA	97.2 ± 7.0	95.5 ± 7.6	95.4 ± 6.2	97.8 ± 5.5	98.4.± 3.0
			*		
LA	97.8 ± 3.6	96.5 ± 4.8	97.8 ± 2.5	98.2 ± 3.3	98.6 ± 2.2
% Extra-calcified eggs					
HA	.14 ± .76	.84 ± 1.79	.61 ± 1.77	.28 ± 1.23	.14 ± .63
LA	.35 ± 1.15	.62 ± 1.86	.64 ± 1.38	.23 ± .80	.13 ± .55
% Double-yolk eggs					
HA	.60 ± 1.61	.54 ± 1.13	.72 ± 1.81	.27 ± 1.05	.26 ± .71
				**	**
LA	.72 ± 1.46	.78 ± 1.81	.59 ± 1.18	1.32 ± 3.05	.63 ± 1.14
% Broken eggs					
HA	.04 ± .38	.40 ± 1.31	.52 ± 1.42	.10 ± .44	.06 ± .33
			*		
LA	.03 ± .27	.47 ± 1.19	.12 ± .44	.05 ± .27	.15 ± .42
% Other defective eggs					
HA	1.52 ± 5.31	2.69 ± 5.94	2.73 ± 4.90	1.54 ± 3.86	1.13 ± 2.54
			**	**	
LA	1.10 ± 2.67	1.61 ± 2.97	.84 ± 1.55	.24 ± .86	.55 ± 1.61

*, ** Lines are significantly different ($p \leq .05$; $p \leq .01$).

Appendix Table 3. Frequency of alleles at alloantigen systems for lines selected for high (HA) or low (LA) antibody response to sheep erythrocytes in generation 10, Chapter III.

	Gene Frequencies[1]						
Allele	*A*	*B*	*C*	*D*	*E*	*H*	*I*
1	17/ 0			29/51	74/88	0/26	
2	24/ 2		40/ 5		26/12	100/74	32/33
3				71/39			
4	59/98		9/ 0	0/10			68/67
5			51/95				
13		15/99					
21		80/ 1					
31		5/ 0					

[1] Gene frequencies (%) for lines HA and LA are indicated to the left and right of the slash (/), respectively. Both lines were L^2L^2.

Appendix Table 4. Frequency of alleles at alloantigen systems for lines selected for high (HA) or low (LA) antibody response to sheep erythrocytes in generation 11, Chapter III.

	Gene Frequencies[1]						
Allele	*A*	*B*	*C*	*D*	*E*	*H*	*I*
1	15/ 0			26/41	71/97	0/36	
2	30/ 1		22/ 2		29/ 3	100/64	29/36
3				74/41			
4	55/99		22/ 0	0/18			71/64
5			56/98				
13		6/99					
21		89/ 1					
31		5/ 0					

[1] Gene frequencies (%) for lines HA and LA are indicated to the left and right of the slash (/), respectively. Both lines were L^2L^2.

Appendix Table 5. Frequency of alleles at alloantigen systems for lines selected for high (HA) or low (LA) antibody response to sheep erythrocytes in generation 12, Chapter III.

	Gene Frequencies[1]						
Allele	*A*	*B*	*C*	*D*	*E*	*H*	*I*
1	18/ 0			21/37	65/94	0/23	
2	34/ 3		20/ 0		34/ 6	100/77	28/36
3				79/31			
4	49/97		25/ 0	0/32	1/ 0		72/64
5			55/100				
13		3/96					
21		96/ 4					
31		1/ 0					

[1] Gene frequencies (%) for lines HA and LA are indicated to the left and right of the slash (/), respectively. Both lines were L^2L^2.

Appendix Table 6. Frequency of alleles at alloantigen systems for lines selected for high (HA) or low (LA) antibody response to sheep erythrocytes in generation 13, Chapter III.

	Gene Frequencies[1]						
Allele	*A*	*B*	*C*	*D*	*E*	*H*	*I*
1	24/ 0			9/37	69/95	0/25	
2	31/ 2		28/ 0		31/ 5	100/75	24/37
3				91/32			
4	45/98		19/ 0	0/31			76/63
5			53/100				
13		1/98					
21		99/ 2					
31							

[1] Gene frequencies (%) for lines HA and LA are indicated to the left and right of the slash (/), respectively. Both lines were L^2L^2.

Appendix Table 7. Frequency of A-E combinations (haplotypes) of lines selected for high (HA) or low (LA) antibody response to sheep erythrocytes, Chapter III.

A-E Haplotype	Gene Frequencies[1] in Generation			
	10	11	12	13
1 1	16/ 0	13/ 0	18/ 0	23/ 0
2 1	2/ 0	1/ 0	0/ 1	1/ 0
2 2	22/ 1	30/ 1	34/ 4	31/ 2
4 1	55/92	55/96	48/94	45/95
4 2	5/ 7	1/ 3	<1/ 1	0/ 3

[1] Haplotype frequencies (%) for lines HA and LA are indicated to the left and right of the slash (/), respectively.

LIST OF TERMS

BSA	bovine serum albumen
C	control treatment
dw	sex-linked dwarf allele
Dw^+	non-dwarf wild type allele
G	line of White Leghorns segregating for 4 MHC haplotypes
GA-5	strain of Marek's disease virus
GAT	polymer of glutamic acid, alanine, and tyrosine
GAT-HI	having high antibody response to GAT
GAT-LO	having low antibody response to GAT
GVH	graft-versus-host
H/L	heterophil to lymphocyte ratio
HA	High Antibody line
HW	High Weight line
HWD	dwarf subline of HW
HWN	non-dwarf HW line
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
i.m.	intramuscular injection
i.v.	intravenous injection
JM-10	strain of Marek's disease virus
LA	Low Antibody line
LPS	lipopolysaccharide
LW	Low Weight line
LWD	dwarf subline of LW
LWN	nondwarf LW line
Ly-4	lymphocyte antigen

M	line of White Leghorns having B^2 at the MHC
MER	mercaptoethanol resistant antibody
MES	mercaptoethanol susceptible antibody
MHC	major histocompatibility complex
N	normal (non-dwarf)
PPI	post primary inoculation
PSI	post secondary inoculation
R	line of White Leghorns having B^{15} at the MHC
RB-1B	strain of Marek's disease virus
SRBC	sheep red blood cells
T_3	triiodothyronine
T_4	thyroxine
TF	thiouracil-fed treatment
Th-1	thymus antigen

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GENETICS, IMMUNORESPONSIVENESS, AND DISEASE RESISTANCE OF CHICKENS

by

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Genetics

(ABSTRACT)

The experiments reported in this dissertation explored the effects of selection for antibody response on other immunological measures and on production traits. The role of thyroid hormones in antibody response was also studied. Selection for high (HA) and low (LA) antibody response to sheep erythrocytes altered subclasses of antibodies in different ways. In line LA antibody response was primarily mercaptoethanol-susceptible (IgM), while the line HA response was primarily mercaptoethanol-resistant antibody (IgG).

Sublines of HA and LA were developed with all possible combinations of major histocompatibility complex haplotypes B^{13} and B^{21}. An experiment was conducted to test Marek's disease resistance of these haplotypes in line LA. Mortality from a natural exposure was high for all three groups, and there was no difference among haplotypes.

Correlated responses of growth and reproductive traits in lines HA and LA were due to genetic correlations with antibody response. These genetic correlations were generally negative and are suggestive of differential allocation of resources. Phenotypic correlations were gener-

ally very small. Changes in allelic frequencies at alloantigen systems were also observed in response to selection.

Experiments designed to study the role of thyroid hormones on antibody responses showed no direct relationship. Chickens from lines HA and LA fed thiouracil exhibited hypothyroidism but did not differ from controls in antibody response. Differences in thyroid hormone concentrations between lines of dwarf and non-dwarf White Rocks selected for high and low juvenile body weight bore no relationship to differences in antibody responses.