ALLOANTIGEN SYSTEMS AND RESISTANCE TO <u>EIMERIA</u> <u>TENELLA</u> AND NEWCASTLE-B1 IN CHICKENS SELECTED FOR RESPONSE TO SHEEP ERYTHROCYTES

by

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INTRODUCTION

Prevention and control of disease are major factors in poultry production. Costs due to mortality, reduced performance, and disease prevention programs contribute to production expenditures, with economic losses estimated at 10 to 15% (Gavora and Spencer, 1983). The cost of anticoccidials alone is estimated at 80 million dollars annually in the USA (Shane, 1985).

Reduction of performance losses can be effected by preventive husbandry procedures such as vaccination programs and feed additives, as well as by breeding for disease resistance. Vaccination programs, however, may lead to reduced genetic potential for disease resistance (Hutt, 1958), and the use of certain additives, some of which are used as coccidiostats, may have adverse effects on growth (e.g., Damron et al., 1977). In cases such as Marek's disease, both husbandry and genetic programs may be necessary for adequate control (Gavora and Spencer, 1978).

In poultry, the genetics of resistance to disease and other stressors is in its infancy when compared to the state of the science in plant breeding. Until recently much of the research involving disease resistance with poultry has emphasized inborn error due to neurological mutants (Siegel, 1979), selection for general immune responses (Siegel and Gross, 1980), and identification and elimination of breeders who are carriers of pathogens (Spencer et al., 1979; Gavora et al., 1980).

The discovery of the major histocompatibility complex (MHC) in chickens by Briles in 1950 and its effects on Mareks disease and Salmonella pullorum infection may be of major importance to breeding for resistance to various pathological conditions. Furthermore, the development of monoclonal and molecular biological procedures may have potential in application of poultry genetics to improvement of germ plasm used in poultry production. Within the period while the experiments discussed in this thesis were conducted, Johnson and Edgar (1984), Clare et al. (1985), and Hartmann et al. (1985) reported that the MHC and other blood allosystems may be involved in resistance to coccidiosis.

The aim of the research presented in this thesis was to further the knowledge of some of the genetic mechanisms involved in resistance to Newcastle disease and cecal coccidiosis.

REVIEW OF LITERATURE

Blood Allosystems and Disease Resistance. The major histocompatibility complex (MHC) of the chicken is located on a microsome and is linked to the nucleolar organizer (Bloom and Bacon, 1985). The B blood group locus, as the MHC of the chicken is known, consists of three regions (Pink et al., 1977). The B-G region codes for antigens found on erythrocytes (Longenecker and Mosmann, 1981) and the B-F region codes for antigens found on erythrocytes and other nucleated cells (Hala et al., 1977; Vainio et al., 1984). The B-L region includes the so-called immune response (Ir) genes (Longenecker and Mosmann, 1981) and codes for antigens found on B cells and macrophages which integrate B-cell, T-cell and macrophage activity (Vainio et al., 1984). The Ir region of the MHC exerts some control over serum levels of immunoglobulin G (IgG) through the Ir-GAT locus (Rees and Nordskog, 1981), a locus specific for immune response to the amino acid polymer Glu-Ala-Tyr which has been given the acronym GAT (Nordskog, 1983).

The B-complex is associated with resistance to lymphoid leukosis (Van der Zijpp, 1983a), immune responses to various antigens and polypeptides (Pevzner et al., 1978; Nordskog, 1983), skin graft rejection (Van der Zijpp, 1983a), tumor regression (Collins et al., 1980), resistance to Marek's disease (Briles et al., 1977; 1980; 1982), and antibody response to Salmonella pullorum (Pevzner et al., 1975). Briles et al. (1977) observed that lines selected for high and low resistance to Marek's disease segregated differently at the B locus. After eight generations of selection, the resistant line was completely homozygous for the B^{21} allele while the susceptible line had a high frequency of the B^{19} allele (freq. = .97) with the remainder being B^{13} (freq. = .03). Subsequent experiments by Briles et al. (1980) indicated that the B^{2} and B^{6} alleles may be involved in resistance to natural outbreaks of Marek's disease.

Leghorns of $B^{1}B^{1}$ genotype were low responders to GAT while $B^{19}B^{19}$ birds were high responders (Pevzner et al., 1978). Rees and Nordskog (1981) found that $B^{1}B^{1}$ birds had higher blood levels of IgG at one year of age than did $B^{19}B^{19}$ birds. Leghorn lines selected for high antibody response to SRBC antigens had a high frequency of B^{21} (freq. = .80) while those selected for low antibody response to SRBC had a frequency of .99 for the B^{13} allele (Dunnington et al., 1984).

The B locus is also implicated in resistance to coccidiosis. Hartmann et al. (1985) found different levels of resistance to coccidiosis among birds with various combinations of alleles at the B locus. Clare et al. (1985) showed that birds of $B^{5}B^{5}$ genotype were more resistant to a challenge with E. tenella than those with $B^{2}B^{2}$ or $B^{2}B^{5}$

combinations.

Blood allosystems other than the B locus are also implicated in disease resistance. Collins _et_ _al_. (1980), using seven highly inbred Leghorn lines, found differences in Rous sarcoma tumor regression in lines homozygous for the B^2 allele. Tumor regression was significantly higher in Line 6_3 than in Line 7_2. These two lines were found to differ at the D, E, I, K, and P loci. Previous studies indicated that the D and I loci were not involved with tumor regression (Collins _et_ _al_., 1977).

Lines selected for high and low antibody response to sheep erythrocytes (Siegel and Gross, 1980) and known to differ in response to various disease antigens (Gross _et_ _al_., 1980) were blood typed at eight blood antigen loci (Dunnington _et_ _al_., 1984). The lines were found to differ in gene frequencies at the A, B, C, D, E, and H loci. Both lines were homozygous for the L^1 allele and had similar gene frequencies at the I locus (freq. I^2 = 33%; freq. I^4 = 67%). At the D locus, the high antibody response (HA) line was 71% D^3 and 29% D^1 while the low antibody (LA) line was 39% D^3, 51% D^1 and 10% D^4. At the C locus, the LA line was 95% C^5 and 5% C^2 while the HA line was 51% C^5, 40% C^2 and 9% C^4 at the time of blood typing.

Johnson and Edgar (1984) showed that the A-E linked systems segregated differently in lines selected for resistance (R) and susceptibility (S) to <u>Eimeria tenella</u>. Genotypes in line R were A^7E^5 and A^9E^1 while those in line S were A^9E^3, A^9E^5, A^9E^2 and $A^{11}E^1$. These lines also differed in plasma levels of corticosterone (Challey, 1966). Gross (1976) showed that a line selected for high plasma corticosterone to social stress was more resistant to challenge with <u>E</u>. <u>necatrix</u> than a line selected for low plasma corticosterone, indicating that stressors may play a role in resistance to coccidiosis.

Schierman and Nordskog (1965) exchanged skin grafts between birds of the same B genotype but differing at the C locus. There were highly significant differences in survival of skin grafts from C incompatible donors and C compatible donors.

<u>SRBC and Disease Resistance</u>. Antibody response to SRBC antigen has been used as a measure of general disease resistance (Van der Zijpp, 1983a) and lines selected for high and low antibody response were developed from a common ancestral Leghorn randombred population (Siegel and Gross, 1980). The line with high five-day antibody titers to SRBC is more resistant to <u>Mycoplasma gallisepticum</u>, <u>Eimeria necatrix</u>, splenomeglia, Newcastle disease, and Northern fowl mite than the low line. In contrast, the low line is more resistant to <u>Staphylococcus aureus</u> and <u>Escherichia coli</u> (Gross <u>et al</u>., 1980). Response to SRBC antigen varies with

age (Siegel et al., 1982; Van der Zijpp, 1982), source of antigen (Van der Zijpp, 1983b), dose (Van der Zijpp, 1983b; Ubosi et al., 1985b), and vaccination for control of various infectious agents (Van der Zijpp et al., 1982). It has also been demonstrated that different temporal relationships exist among populations for primary and secondary immunizations with SRBC (Ubosi et al., 1985b; Van der Zijpp, 1983b).

2-Mercaptoethanol resistant (MER) titers are used as an indicator of relative levels of IgG in an antibody response (Siegel et al., 1983). MER antibody response to SRBC does not necessarily follow the same patterns of response as total antibody. Van der Zijpp and Leenstra (1980) showed that the MER response was somewhat delayed in relation to the total antibody response. The exact temporal patterns vary with genetic stock and dose (Van der Zijpp, 1983b) as well as between primary and secondary immunizations (Ubosi et al., 1985b; Van der Zijpp et al., 1983). The proportion of total SRBC titers due to MER antibody is affected by strain, dose, primary vs secondary response (Yamamoto and Glick, 1982; Van der Zijpp, 1983b; Ubosi et al., 1985b), and by mild stressors such as vaccination (Van der Zijpp et al., 1982) and exposure to cold (Subba Rao and Glick, 1977).

Although no differences were found in hemagglutination inhibition (HI) titers to attenuated or inactivated

Newcastle disease virus (NDV) in populations as widely divergent as Bedouin fowl and commercial Leghorn chickens (Heller _et al_., 1981), genetic differences in immune response to Newcastle vaccine were reported among broiler stocks (Siegel _et al_., 1984). Genetic variation within a population may be moderate to high, as evidenced by heritabilities between .14 and .60 for antibody titers to Newcastle virus (Peleg _et al_., 1976; Soller _et al_., 1981; Van der Zijpp, 1983a). Genetic differences to Newcastle virus, however, may be influenced by dosage (Siegel _et al_., 1984), which in turn could influence heritability estimates. Thus it is important to consider route of administration, dosage, and other variables in estimation of heritability of antibody titers. This statement is made recognizing fully that heritability estimates are for specific populations at particular points in time.

Soller _et al_. (1981) inoculated Leghorn and White Rock chicks with inactivated Newcastle virus and/or _Escherichia coli_ vaccine. Although HI titers for Newcastle given alone were significantly different from those of birds vaccinated with both antigens, there was no consistent pattern. Van der Zijpp (1983a) reported genetic correlations between HI and SRBC titers ranging from .53 to -.54. Earlier, Frankena (1980) reported genetic correlations between these two titers of .18 and .43, depending on what day post-inoculation with SRBC the blood was taken. Lower mortality and higher HI titers were observed to a challenge with Newcastle in the third generation of a line selected for high antibody response to SRBC than in one selected for low antibody response (Gross et al., 1980). The varying results observed in Newcastle response when given in conjunction with other antigens infers that response may be enhanced or masked depending on the specific setting. Clarification of the genetic and environmental correlations requires further investigation.

Though the benefits of genetic resistance to diseases such as Newcastle are obvious, very highly resistant birds are not necessarily more desirable to the producer than very susceptible birds. The allocation of resources toward immune responses detracts from growth and reproductive traits (Siegel et al., 1982) and thus selection for genetic resistance to diseases should be implemented into breeder improvement programs with this in mind.

EXPERIMENT I

GENETIC RESISTANCE TO *EIMERIA TENELLA*

INTRODUCTION

Selection and crossing experiments conducted decades ago demonstrated additive genetic variation in chickens for resistance to cecal and intestinal coccidiosis (e.g., Edgar *et al*., 1951; Champion, 1954; Rosenberg *et al*., 1954). Concomitant research contributed to the use of feed additives and vaccines for control of coccidia in poultry. The modest success of these two venues probably reduced interest in developing genetic approaches for resistance to these protozoa. Nongenetic control of coccidiosis, however, has disadvantages which include reduced feed consumption (e.g., Gard *et al*., 1975; Damron *et al*., 1977), natural selection for coccidia resistance to additives (see review by Chapman, 1982), and increased processing problems (e.g., Angel *et al*., 1985).

Lines of chickens developed for resistance and susceptibility to *Eimeria tenella* differed in plasma corticosterone levels, with the former having higher values than the latter (Challey, 1966). Also, a line selected for high plasma corticosterone level to social strife was more resistant to a challenge with *Eimeria necatrix* than one selected for low corticosterone response (Siegel, 1976). Thus, the relationship between responses to stressors and coccidiosis resistance may be of commercial importance with mild stressors enhancing resistance (Gross, 1976). There is also recent evidence suggesting that certain blood allosystems may be involved with resistance to coccidia. Lines of chickens selected for resistance and susceptibility to _E. tenella_ differ in frequencies of alleles for the A-E linked blood allosystem loci (Johnson and Edgar, 1984). Genotypes of chickens from the resistant line were A^7E^5 and A^9E^1 while those from the susceptible line lacked the A^7 allele and were A^9E^3, A^9E^5, A^9E^2, and $A^{11}E^1$. Hartmann _et al_. (1985) observed differences in mortality from coccidiosis among allelic combinations at the B locus, while Clare _et al_. (1985) reported that B^5B^5 chickens were more resistant to a challenge with _E. tenella_ than those with B^2B^2 or B^2B^5 genotypes. The experiments reported here explore the role of genetic variation and specific blood allosystems in resistance to natural and controlled exposures to _E. tenella_.

MATERIALS AND METHODS

Populations used in these experiments were the tenth and eleventh generations of lines selected for high (HA) and low (LA) antibody response to sheep red blood cells (Siegel and Gross, 1980). These lines have been blood typed for eight alloantigen systems and differ in allelic frequencies at some loci (Dunnington *et al*., 1984). Matings to produce progeny for these experiments were made so that line HA progeny were homozygous B^{21}, H^2, and L^1. In line LA, homozygosity occurred for: A^4, B^{13}, C^5, E^1, H^2 and L^1. Matings were to obtain segregants at loci C, D, and I in line HA and D and I in line LA. All chicks were pedigreed and vaccinated for Marek's disease at hatch.

Natural outbreaks.

Two trials involving natural exposure to coccidiosis were conducted. In each trial, chicks of age-contemporary parents from both lines were hatched, raised in floor pens with wood shavings, and fed a diet containing the anticoccidial furazolidone, NF-180 (Reid *et al*, 1984). Autopsies of dead birds showed mortality in all trials was due to *E. tenella*.

Trial one. Chicks from lines HA (n=107) and LA (n=153) were raised in pens with used wood shavings seeded with *E. tenella* from a previous outbreak. In line HA, males of C^2C^2 and C^5C^5 genotypes were each mated to C^2C^2, C^2C^5, and C^5C^5

females. Matings involving loci D and I were:

D^1D^3 x D^1D^3, D^1D^3 x D^3D^3, D^3D^3 x D^3D^3

I^2I^2 x I^2I^2, I^2I^2 x I^2I^4, I^2I^2 x I^4I^4,

I^2I^4 x I^2I^4, I^2I^4 x I^4I^4, I^4I^4 x I^4I^4.

In line LA, D^1D^1 and D^3D^3 males were mated to D^1D^1, D^1D^3, and D^3D^3 females. Matings involving locus I were: I^2I^2 x I^2I^4, I^2I^2 x I^4I^4, I^2I^4 x I^2I^4, I^2I^4 x I^4I^4, I^4I^4 x I^4I^4.

When mortality was observed, sulfaquinoxaline was added to the drinking water for three consecutive days. Mortality was recorded to 15 days of age. Chi-square (X^2) tests were performed to determine if there were differences in mortality due to lines, sire groups within lines, and alleles at loci C, D, and I within lines. When significant X^2 values were found, Bonferroni Chi-square tests were conducted within groups (Jensen et al., 1968).

Trial two. Line HA and LA birds were raised in floor pens with used wood shavings. Matings involving locus C in line HA were such that each C^2C^2 or C^5C^5 sire was mated to C^2C^2, C^2C^5, and C^5C^5 dams. Matings for loci D and I were:

D^1D^3 x D^1D^3, D^1D^3 x D^3D^3, D^3D^3 x D^3D^3

I^2I^2 x I^2I^4, I^2I^4 x I^2I^4, I^2I^4 x I^4I^4, I^4I^4 x I^4I^4

In line LA, D^1D^1 and D^3D^3 sires were each mated to D^1D^1, D^1D^3, or D^3D^3 dams, and matings involving locus I were:

I^2I^2 x I^2I^2, I^2I^2 x I^2I^4, I^2I^2 x I^4I^4,
I^2I^4 x I^2I^4, I^2I^4 x I^4I^4, I^4I^4 x I^4I^4.

Exposure to E. tenella was through transmission by caretakers working with other infected birds in the same building. Chick numbers before coccidiosis was observed were 182 and 217 in lines HA and LA, respectively. At the time mortality was first observed, all birds were treated with sulfaquinoxaline in drinking water for three consecutive days. As this procedure was not effective in curbing mortality, the treatment was repeated seven days later. Mortality was recorded to 64 days of age. Chi-square tests to determine differences in mortality due to line, sires within lines, and alleles at loci C, D, and I were performed within lines at 31, 38, 45, 51, and 64 days of age. When significant X^2 values were found, Bonferroni Chi-square tests were conducted within groups.

Controlled challenge

Chicks used in this experiment were reared in battery brooders with raised wire floors and fed a diet without an anticoccidial agent. In line HA, matings involving locus C were:

C^2C^2 x C^2C^2, C^2C^2 x C^2C^5, C^2C^2 x C^5C^5,
C^5C^5 x C^2C^5, and C^5C^5 x C^5C^5.

Those for the D locus were:

$D^1D^3 \times D^1D^3$, $D^1D^3 \times D^3D^3$, $D^3D^3 \times D^3D^3$

and those for the I locus were:

$I^2I^2 \times I^2I^4$, $I^2I^4 \times I^2I^4$, and $I^2I^4 \times I^4I^4$.

In line LA, all chicks were typed for alleles at loci D (D^1D^1, D^1D^3, and D^3D^3), and I (I^2I^2, I^2I^4 and I^4I^4).

Procedures and dosages for the control challenge were developed from preliminary experiments with these lines. On the basis of these results, each chick was inoculated orally at 20 days of age with 460 oocysts of E. tenella. Body weights were obtained at 31 days of age when chicks were challenged orally with 8000 oocysts of E. tenella, and blood was obtained from each LA line chick to determine alleles at loci D and I. Six days after challenge (37 days of age), birds were weighed, killed by cervical dislocation, and ceca examined macroscopically and scored for lesions. Scores were:

0 -- No visible lesions

1 -- Slight thickening of the cecal wall

2 -- Moderate thickening of cecal wall; no blood visible

3 -- Moderate to severe thickening of cecal wall; moderate blood in cecum

4 -- Cecum filled with blood

Relative weight change was calculated as the difference between body weight at the age (31 days) of challenge and when lesion scores were determined (37 days), divided by the weight at the time of challenge. Relative weight changes were transformed to arc sine square roots and lesion scores to square roots prior to analyses of variance to determine effects of line, sex, sire, and either dam within sire or genotypes within sire at the C, D, and I loci.

RESULTS

Natural Outbreaks.

Trial one. Mortality due to cecal coccidiosis was first observed at 11 days of age and by 15 days of age had reached 12% in line HA and 29% in line LA. This difference between lines was highly significant. Differences in mortality were not significant for sire or genotype in line LA (Table 1). In line HA, however, mortality was significantly greater among progeny from D^1D^3 x D^1D^3 (36%) matings than from D^1D^3 x D^3D^3 (8%), or D^3D^3 x D^3D^3 (10%). Differences among sires or alleles at other loci were not significant in line HA.

Trial two. Mortality in line LA birds was first observed at 28 days of age, reached 41% by 38 days and 77% by 51 days (Table 2). In contrast, only 8% of the HA birds had died by 38 days of age, and most mortality in this line occurred after that age. The difference in total mortality between lines was highly significant, being 65% for line HA and 86% for line LA. Although data were obtained to 64 days of age, there were no further deaths after 58 and 52 days of age in lines HA and LA, respectively.

Chi-square analyses for mortality showed no significant differences involving matings at loci C and D (Table 2), while differences were found at locus I in both lines. More detailed analysis of those matings which produced offspring of known genotypes showed that mortality due to genotypes at the I locus also occurred at younger ages in line LA than HA (Table 3). Within line comparisons revealed lower mortality in line LA for I^4I^4 chicks than for those with other alleles at this locus (Figure 1). In line HA, mortality was significantly lower in chicks from I^2I^4 x I^4I^4 matings than the other two matings.

Although there appeared to be a trend for lower mortality from D^1D^3 x D^1D^3 matings than for either D^1D^3 x D^3D^3 or D^3D^3 x D^3D^3 matings in line HA, and from D^1 D^1 x D^1D^3 than from D^1D^1 x D^1D^1, D^1D^1 x D^3D^3, or D^1D^3 x D^3D^3 matings in line LA, differences were not significant. No effect of alleles at locus C was observed in line HA.

Controlled Challenge

Lesions. Since there was no significant sexual dimorphism for lesion scores, data for both sexes were pooled for subsequent analyses. The proportion of responders (chicks exhibiting macroscopic lesion scores of 1 to 4) was significantly higher in line LA than in line HA (Table 4). Although lesion scores were also significantly greater for line LA than for HA chicks, this difference disappeared when chicks not exhibiting lesions were omitted from the analysis.

Significant differences among sire families for lesion scores were observed in line HA (Table 5) but not LA.

Although there were no differences among sire families in frequency of responders, rank correlation between mean score and percent responders was 1.00.

There were no significant differences in lesion scores due to genotype within sires within lines at loci C, D, or I. When variation due to genotype was analyzed across sires, significant differences were found at locus I in line HA with chicks from I^4I^4 homozygous parents showing greater resistance as measured by lesions than those from I^2I^4 x I^4I^4 or I^2I^4 x I^2I^4 matings (Table 6).

<u>Body weights</u>. There were significant differences between lines and between sexes for body weight (Table 7). Line LA chickens were heavier than those from line HA and males weighed more than females. The lack of a significant line by sex interaction indicated similar sexual dimorphism in both lines. Alleles at loci C, D, and I had no significant effect on body weight.

Relative changes in body weight after a challenge with coccidia can be used as an assay of host response (e.g., Giambrone <u>et</u> <u>al</u>., 1984). Accordingly, relative gain from 31 to 37 days of age was used to measure response to the <u>E. tenella</u> challenge. Since there were line differences for lesion scores depending on whether chickens with or without macroscopic lesions were included in the analyses, this variable was included along with line and sex as a main effect in the analysis of variance. Relative changes in weight were significantly less for females than for males and for line LA than HA (Table 7). Also, birds showing macroscopic lesions had a significantly smaller relative weight changes (26.7 ± .3) than those without lesions (28.4 ± .4). There were no significant interactions between any of the main variables.

To examine further the lesion-weight change relationship, comparisons of sire families and genotypes were made within each line. In line HA, sire family 853 had significantly lower lesion scores and greater relative weight gain than families 813 and 834 (Table 5). When relative gain was compared among genotypes at the I locus, trends, while following those for lesion scores (Table 6), were not significant. In line LA, where there were no differences among sire families or at locus I for lesion scores, differences in relative gain were significant. Relative gain of all birds was 26.5 ± .9ab, 25.2 ± .5b, and 27.6 ± .5a for genotypes I^2I^2, I^2I^4, and I^4I^4, respectively, demonstrating greater resistance to <u>E</u>. <u>tenella</u> by I^4I^4 chicks. Thus, the I^4 effect on resistance was more evident in relative weight gain in line LA and in lesion scores in line HA.

DISCUSSION

Previous experiments showed that line LA chicks weighed more than those from line HA (Siegel _et al_., 1982; Ubosi _et al_., 1985). Also, line HA chickens were more resistant to Newcastle disease, northern fowl mite, _E_. _necatrix_, and _Mycoplasma gallisepticum_, and less resistant to _Escherichia coli_ and _Staphylococcus aureus_ than those from line LA (Gross _et al_., 1980). Comparisons presented here show that chicks from line HA were more resistant than those from line LA to both natural outbreaks and a controlled challenge of _E_. _tenella_. Line differences may be due in part to a correlated response to selection for response to SRBC antibody and/or to allotypes at the B locus since birds were homozygous for B^{21} in line HA and B^{13} in line LA. The B^{21} allele confers resistance to Marek's disease (Briles _et al_., 1977) and other alleles at this locus influence mortality from Marek's disease and coccidiosis (Hartmann _et al_., 1985), and resistance to _E_. _tenella_ (Clare _et al_., 1985). The data presented here, plus those from other laboratories (Johnson and Edgar, 1984; Clare _et al_., 1985; Hartmann _et al_., 1985) suggest involvement of several allosystems in response of chickens to coccidia.

Differences in lesion scores and relative weight change between lines resulted more from the number of individuals exhibiting macroscopic lesions (HA < LA) than from severity of the lesions, suggesting a higher threshold for susceptibility to E. tenella in HA than LA birds. This reasoning is consistent with the procedure whereby differences in thresholds between lines susceptible and resistant to E. tenella were circumvented in controlled challenges by altering the number of oocysts inoculated (Giambrone et al., 1984).

The data suggested that, in lines relatively resistant to E. tenella, frequency of individuals exhibiting macroscopic lesions and their lesion scores are more effective assays of a challenge than relative change in weight after challenge. In more susceptible lines, relative change in weight and mortality are better assays because the severity of response can mask lesion scores. Threshold differences can contribute to asymmetry in responses noted in long term selection for resistance and susceptibility to E. tenella (Johnson and Edgar, 1982). In humans, thresholds to specific diseases may be affected by family group since the liability to diseases such as diabetes mellitus, renal calculi, and peptic ulcers is greater in relatives of affected individuals than for the population as a whole (Falconer, 1965; 1967).

In natural outbreaks and a controlled exposure of E. tenella, severity of response was influenced by alleles at the I locus in both lines. The primary effect of I alleles appeared as a change in threshold for susceptibility, as evidenced by a change in the number of responders in the challenge experiment. When the I^4 allele was in the homozygous state, there was a reduction in the magnitude of response as well as a change in threshold. Overall mean scores at the I locus had a linear relationship, which indicated genetic additivity depending on the number of I^4 alleles. Mean scores were 1.71, 1.42, and 0.98 for I^2I^4 x I^2I^4, I^2I^4 x I^4I^4, and I^4I^4 x I^4I^4, respectively. Additivity was also noted at the I locus in a natural outbreak when line LA birds of I^2I^2 x I^2I^2, I^2I^4 x I^2I^4 and I^4I^4 x I^4I^4 matings were compared.

These results were consistent with those showing additive effects of allele B^{21} on resistance to Marek's disease (Briles *et al*., 1977). The effects of locus I on response to *E. tenella* may not be seen if exposure is mild (Trial 1) or the effects may be overridden with severe exposure (Trial 2). Thus, comparisons involving resistance to *E. tenella* are influenced by the background genome and severity of the challenge.

SUMMARY

Lines selected for high (HA) and low (LA) antibody response to sheep erythrocytes were used to study relationships between blood allosystems and resistance to *Eimeria tenella*. Results of natural and controlled exposures to *E. tenella* followed the same pattern with line HA chickens being more resistant than those from line LA. Differences between lines may be due, in part, to effects of the major histocompatibility complex (MHC) since line HA was $B^{21}B^{21}$ and line LA was $B^{13}B^{13}$. No difference in resistance to *E. tenella* was found among genotypes at the C locus, while influences of alleles at the D locus were inconclusive. Alleles at locus I, however, had definite effects on resistance in both the natural exposure and controlled challenge, with allele I^4 conferring more resistance than I^2. Results showed further that genetic resistance to cecal coccidiosis is affected by severity of exposure as well as background genome.

Table 1. Chi-square (X^2) values for _E_. _tenella_ mortality comparisons of sires and genotypes within lines in Trial 1

Category	HA		LA	
	df	X^2	df	X^2
Sire	3	2.27	3	5.56
D locus	2	8.47*	4	4.38
I locus	5	3.42	4	1.71
C locus	4	3.62	-	--

*P < .05.

Table 2. Mortality by lines and Chi-square (X^2) values for *E. tenella* mortality comparisons of genotypes within lines at several ages in Trial 2

Days of age	% mortality HA	% mortality LA	X^2 values at loci: HA line C	HA line D	HA line I	LA line D	LA line I
31	4.4	28.0	3.17	2.53	1.46	3.43	8.46
38	7.7	41.0	1.15	2.55	2.00	2.77	10.37†
45	15.4	57.1	3.73	1.41	5.26	3.02	12.37*
51	24.2	77.0	2.53	1.54	7.29†	0.72	4.16
64	64.8	85.7	7.04	2.76	12.84*	0.82	1.69
df			4	2	3	4	5

*$P < .05$, † $P < .07$.

Table 3. Number of birds (n) and Chi-square (X^2) values for *E. tenella* mortality comparisons of genotypes within lines in Trial 2

	HA			LA	
Days of age	C	D	I	D	I
n	98	182	168	122	71
31	2.55	2.53	.47	.66	7.26*
38	.40	2.55	.49	.30	8.58*
45	1.84	1.41	2.34	3.20	9.50**
51	.85	1.54	2.32	.61	1.85
64	.44	2.76	12.55**	.11	.74
df	2	2	2	2	2

HA matings:

C^2C^2 x C^2C^2, C^2C^2 x C^5C^5, C^5C^5 x C^5C^5
D^1D^3 x D^1D^3, D^1D^3 x D^3D^3, D^3D^3 x D^3D^3
I^2I^4 x I^2I^4, I^2I^4 x I^4I^4, I^4I^4 x I^4I^4

LA matings:

D^1D^1 x D^1D^1, D^1D^1 x D^3D^3, D^3D^3 x D^3D^3
I^2I^2 x I^2I^2, I^2I^4 x I^2I^4, I^4I^4 x I^4I^4

*$P < .05$, **$P < .01$.

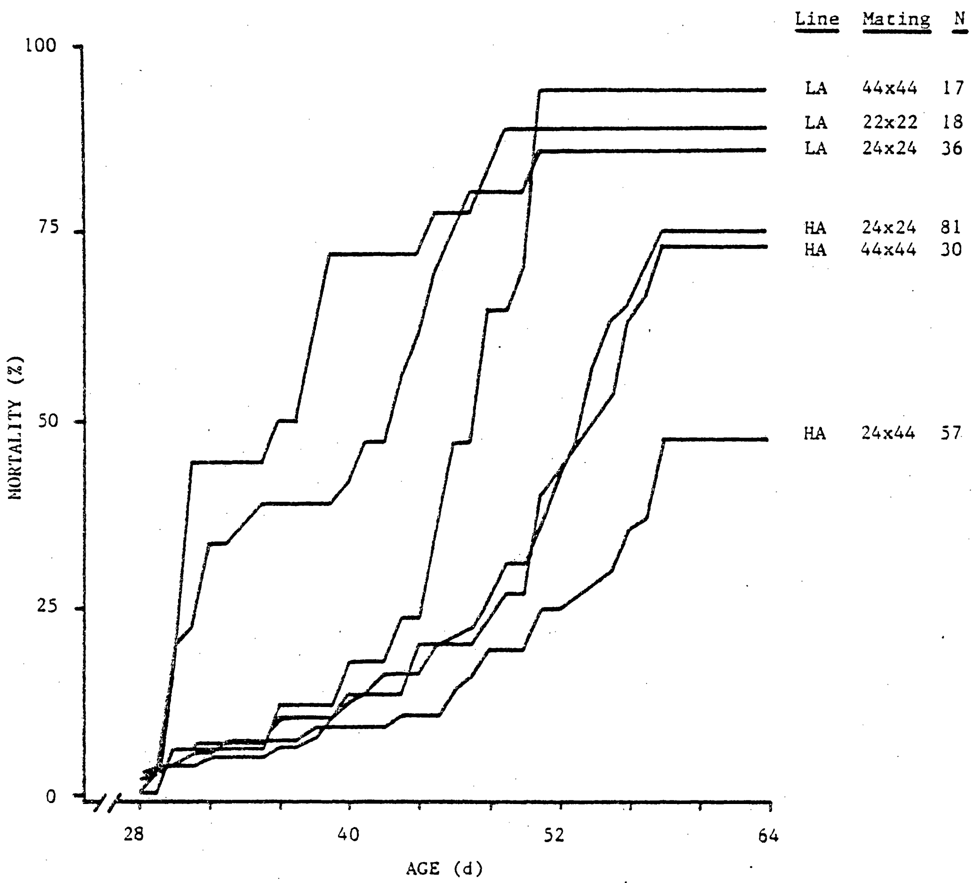

Figure 1. Cumulative mortality by mating at locus I within lines in Trial 2.

Table 4. Responders and lesion scores[1] from a controlled challenge with *E. tenella* by line

Line	n	Responders[2] n	Responders[2] %	Means ± s.e. all	Means ± s.e. responders
HA	217	139	65b	1.42 ± .09b	2.20 ± .09a
LA	263	210	80a	1.75 ± .07a	2.18 ± .06a

[1]Lesion scores analyzed as square roots of actual values.

[2]Chi-square test conducted on actual values of chicks which had macroscopic lesions.

Values in a column with the same letter are not different ($P > .05$).

Table 5. Responders, lesion scores, and percent change in body weight[1] from a controlled challenge with *E. tenella* by sire family in line HA

		Res-ponders[2]		Means ± s.e.			
				Lesion scores		Wt. change (%)	
Sire	n	n	%	all	responders	all	responders
813	58	42	72a	1.67 ± .17a	2.31 ± .15a	27.3 ± .7b	27.5 ± .9a
834	50	35	70a	1.62 ± .19a	2.31 ± .17a	27.5 ± .5b	27.2 ± .6a
901	48	30	62a	1.46 ± .21ab	2.33 ± .21a	26.9 ± .6b	26.7 ± 1.0a
853	60	32	53a	0.97 ± .15b	1.81 ± .16a	29.7 ± .7a	28.9 ± .9a

[1]Analyses of variance conducted on square roots of lesion scores and on arc sin of weight changes.

[2]Chi-squares conducted on actual values, Tau = 3.

Values in a column with the same letter are not different (P > .05).

Table 6. Responders, lesion scores, and percent change in body weight[1] from a controlled challenge with *E. tenella* by genotype at the I locus in line HA

Mating	n	Responders[2] n	Responders[2] %	Means ± s.e. Lesion scores all	Means ± s.e. Lesion scores responders	Means ± s.e. Wt. change (%) all	Means ± s.e. Wt. change (%) responders
$I^2I^4 x I^2I^4$	99	73	74a	1.71 ± .14a	2.32 ± .12a	27.3 ± .4a	27.4 ± .9a
$I^2I^4 x I^4I^4$	62	38	61ab	1.42 ± .17ab	2.32 ± .16a	28.1 ± .7a	27.5 ± .9a
$I^4I^4 x I^4I^4$	47	25	53b	0.98 ± .17b	1.84 ± .20b	29.0 ± .7a	28.2 ±1.0a

[1]Analyses of variance conducted on square roots of lesion scores and on arc sin of weight changes.

[2]Analysis conducted on actual values, Tau = 2.

Values in a column with the same letter are not different (P > .05).

Table 7. Means ± s.e. for actual and relative change in body weight (g) at 31 and 37 days of age from a controlled challenge with *E. tenella*

Group		n	Body weight[1] 31d	37d	Change[2]
Sex	F	218	236 ± 2	296 ± 2	25.8 ± .4
	M	252	277 ± 2	355 ± 3	28.3 ± .3
Line	HA	208	245 ± 3	314 ± 3	27.9 ± .3
	LA	262	267 ± 2	339 ± 3	26.5 ± .3

[1]Analyses of variance for body weight were conducted on common logarithms and those for change in weight on arc sin square roots. Line and sex differences were significant in all comparisons.

[2]Relative change = $\frac{\text{wt 37d - wt 31d}}{\text{wt 31d}} \times 100$

EXPERIMENT II

GENETIC RESISTANCE TO NEWCASTLE-B1 VIRUS

INTRODUCTION

Respiratory infections, long a plague to the poultry industry, continue to be one of the major disease entities in production programs. Newcastle disease, in its various forms, is one of the more important respiratory diseases and procedures for its control include eradication of infected flocks, sanitation measures, and various vaccination programs. There is not only a dearth of information on genetic approaches to control Newcastle disease, but experimental results have been equivocal. For example, although genetic variation in immune response to Newcastle vaccine was observed in chicks inoculated intramuscularly with attenuated or inactivated virus (Peleg et al., 1976; Soller et al., 1981; Van der Zijpp, 1983a), stocks as widely divergent as Bedouin fowl and commercial Leghorns did not differ (Heller et al., 1981).

Investigations measuring the relationships between Newcastle disease and general immunological responses such as antibody titers to sheep erythrocytes have been inconsistent. For example, Frankena (1980) reported positive correlations between antibody titers to sheep erythrocytes and those for Newcastle disease. Yet in a later report, Van der Zijpp (1983a) reported variable correlations for these antibody titers. In comparing lines divergently selected for antibody to sheep erythrocytes, Gross et al. (1980) found lower mortality and higher antibody titers to Newcastle virus in a line selected for high antibody to sheep erythrocytes. Similarly, Van der Zijpp (1984) reported higher HI titers in a high antibody response line to sheep erythrocytes than in the low line.

The experiments reported herein were undertaken to explore possible differences for antibody response to Newcastle disease vaccine between lines of chickens selected for high and low response to sheep erythrocytes, and the magnitude of correlations between titers to these two antigens.

MATERIALS AND METHODS

All chicks used in this experiment were from lines selected for high (HA) and low (LA) production of antibody to sheep red blood cells (Siegel and Gross, 1980). In each trial, chicks from eleventh generation matings within each line were hatched as contemporaries, wingbanded, and reared intermingled in floor pens with wood shavings litter. Feed and water were provided _ad libitum_.

Inoculations of sheep red blood cell (SRBC) antigen consisted of an intravenous dose of 0.1 ml of 0.25% suspension in physiological saline. SRBC antibody was determined by the microtitration method (Wegmann and Smithies, 1966) on blood obtained from the brachial vein and expressed as $\log_2$ of the reciprocal of the highest dilution showing a visible agglutination.

Newcastle disease virus (NDV) was given intranasally as one drop of a 10^{-6} dilution of Newcastle-B1 virus. Antibody to NDV was determined by hemagglutination inhibition (HI) titers (Beard, 1975) from blood obtained from the brachial vein. HI titers were expressed as $\log_2$ of the reciprocal of the highest dilution showing inhibition of agglutination.

Trial one. Male chicks produced from matings of eight HA and six LA line sires to dams from their respective lines were randomized within sire families into two groups and administered antigens as follows:

(a) Treatment S - SRBC was administered to 23 HA and 13 LA line cockerels at 72 days of age.

(b) Treatment N - Newcastle virus was given to 39 HA and 17 LA line cockerels at 63 days of age.

At 77 days of age, blood was obtained for measuring SRBC antibody on all 92 birds and HI titers on the 56 which had received NDV. In addition, seven chicks from these lines which had not been administered either antigen were bled and both SRBC and HI titers determined. Chi-square (X^2) values were calculated to compare lines for frequency of individuals which did and did not produce SRBC and/or HI antibody titers. Analyses of variance were conducted within treatments to determine if line differences existed for SRBC or HI titers. Analyses were made which included all individuals and only those who produced the respective antibody. Sire families were ranked according to mean titers for SRBC and HI and rank correlations were calculated within lines.

Trial two. Cockerels produced from matings of eight HA and eight LA line sires to dams from their respective lines were vaccinated against Marek's disease and randomized within sire families into two treatment groups (S and SN):

(a) Treatment S - SRBC antigen was given to 60 HA and 52 LA line chicks at 43 days of age.

(b) Treatment SN - Newcastle-B1 and SRBC antigen were administered at 34 and 43 days of age, respectively, to 47 HA and 50 LA line chicks.

At 48 days of age, blood samples were taken from the brachial vein of each chick. SRBC antibody titers were determined for all birds. Relative levels of 2-mercaptoethanol resistant (MER) antibodies to SRBC were assayed by the method of Delhanty and Solomon (1966) and expressed similarly to total SRBC titers. The assumption was made that MER represented IgG, although there may be slight differences from the mammalian class (Siegel et al., 1983). HI titers were obtained for those chickens which had received NDV and a sample of 10 males per line from Treatment S to test for cross reactivity of antibodies. All chicks were assayed for SRBC antibody.

Chi-square values were calculated to compare lines for frequency of individuals which did and did not produce SRBC and/or HI titers. Analyses of variance were conducted within treatments to determine if line differences existed in SRBC, MER, and HI titers. Analyses were made for all individuals and for only those who produced the respective antibody. Within line correlations between these titers were calculated for SN birds. Sire families were ranked according to mean SRBC titers for S chicks and mean titers for SRBC and HI for SN birds. Rank correlations were calculated within lines.

RESULTS

Trial One.

Treatment S. Chicks from line HA had significantly higher SRBC titers than those from line LA (Table 8), regardless of whether the analysis was conducted for all birds or for only those which produced antibody. There was no difference between lines for frequency of birds which produced antibody to SRBC antigen.

Treatment N. Although there were no differences between lines in frequency of birds producing antibody to NDV or of titers for chicks administered NDV, when means of responders only were analyzed titers for line LA chicks were significantly higher than those for line HA (Table 9). Rank correlations between sire families within lines for SRBC to HI titers were not significant, being $r = -.18$ in line HA and $r = -.10$ in LA.

Six of seven birds which had not received NDV antigen reacted positively to HI tests. The mean for these chicks was $3.71 \pm .68$. In addition, two birds had titers to SRBC, with values of 3 and 4.

Trial Two.

Treatment S. A greater frequency of cockerels from line HA than LA produced SRBC and MER antibody (Table 10). Analyses of variance showed that HA chicks had significantly higher SRBC and MER titers than those from line LA regardless of whether the analysis was conducted on all chicks or on those that produced antibody to SRBC. The correlation between SRBC and MER was highly significant in line HA (r=.68). This correlation could not be calculated in line LA since only one bird produced MER antibody.

Birds from Treatment S on which HI tests were conducted reacted positively, having an overall mean of 4.60 ± .22. Means within lines were 4.40 for HA and 4.80 for LA with the difference not significant.

Treatment SN. All birds receiving both SRBC and Newcastle antigen produced HI antibody, while the frequency of chicks producing antibody to SRBC was greater in line HA than LA (Table 11). Analyses of variance conducted on data from chicks that received both antigens revealed contrasting differences between lines for SRBC and HI titers in that those from line HA had higher SRBC titers and lower HI titers than those from line LA. When only responders to SRBC were analyzed there were no significant differences between lines for SRBC or HI titers.

Correlations between SRBC, MER, and HI titers in treatment SN varied according to line. In line HA, correlations of SRBC with MER (.46) and with HI titers (.38) were positive and highly significant while that between HI and MER titers (.24) was not significant. Since only one bird produced MER in line LA, the only correlation calculated was that between SRBC and HI titers. This correlation of -.18 was not significant.

Rank correlations of sire families for SRBC titers between group S and group SN were not significant in either line (r=.24 in HA and .14 in LA). When rank correlations of sire families were calculated between SRBC and HI titers in group SN, the correlation in line HA (r=.83) was significant while in line LA (r=.11), it was not significant. The rank correlations of sire families in Treatment S between SRBC and HI titers were not significant in either line, being r = .29 in HA and r = .17 in LA.

DISCUSSION

The higher SRBC titers of line HA than LA chicks found in these trials were in keeping with those reported previously (Siegel and Gross, 1980; Gross et al., 1980; Ubosi et al., 1985a, 1985b). The lower threshold of HA than of LA chicks in responding to SRBC antigen at the dosage used was consistent with results of Ubosi et al. (1985a, 1985b), who showed that differences between populations could be masked at higher dosages of antigen. Ubosi et al. (1985b) also observed higher MER titers in line HA than LA and positive correlations between SRBC and MER titers. Although results obtained here were consistent with those of Ubosi et al. (1985b) and also showed that the relationship existed when Newcastle antigen was given, they should be interpreted with caution since the literature is inconsistent regarding the proportion of SRBC titers due to MER antibodies (Subba Rao and Glick, 1977; Yamamoto and Glick, 1982; Van der Zijpp, et al., 1982, 1983; Van der Zijpp 1983b). Relative levels of MER antibodies are affected by dosage, days post-immunization before tests are conducted, vaccination program, and stressors.

Van der Zijpp and Leenstra (1980) observed the kinetics of total and MER resistant antibodies to SRBC over a period of 13 days following inoculation. Their findings demonstrated that relationships between them change dramatically depending on how long after inoculation titers are determined. Van der Zijpp (1983b) also found that relationships may vary with population. Furthermore, Van der Zijpp et al. (1983) noted different patterns of MER response between primary and secondary inoculations with SRBC, as did Ubosi et al. (1985b) using lines HA and LA.

Hemagglutination inhibition titers to NDV were greater in line LA than in HA. These results were inconsistent with those noted by Gross et al. (1980) with a Newcastle-B1 challenge in the third generation. This discrepancy may be due to a dosage, age or generation effect, or to the difference in measurement criteria since mortality and morbidity were used as indicators of susceptibility in the earlier report.

Differing trends seen when NDV is administered alone versus when it is given to chickens which receive SRBC may indicate interference or enhancement of HI titers by SRBC antibodies. Van der Zijpp, et al. (1982) found that SRBC immunization increased HI titers to NDV vaccine in chickens vaccinated against Marek's disease, infectious bronchitis, and infectious bursal disease. SRBC immunization did not affect HI titers of unvaccinated birds. Results from trials in this study may have been affected by vaccination against Marek's disease, as well as inoculation with SRBC, since Trial 1 chicks were not vaccinated against Marek's but Trial

2 chicks were, possibly enhancing the effects of SRBC on HI titers.

Because the trials conducted in this experiment raise many questions, elucidation of genetic resistance or susceptibility to Newcastle disease must be pursued. Data obtained from uninoculated birds indicate that the presence of low level infection spread by horizontal transmission may be a factor in genetic selection for resistance, the effectiveness of vaccination programs, and the level of titers in flocks. This conundrum suggests that routine husbandry should include screening for presence and level of antibodies to NDV prior to and post-inoculation at periodic intervals. It is not unreasonable that statistical sampling procedures could be developed for routine use not only by primary breeders but in breeding and laying flocks.

Criteria for measuring antibody levels to Newcastle virus were discussed in detail by Beard and Hanson (1984). Moreover, the very recent influx of tests such as fluorescent antibody (FA) and enzyme linked immuno-sorbent assays (ELISA) suggest that usefulness of these procedures to determine antibody levels to NDV should be tested by comparing results to traditional measurements of antibodies such as HI tests. Preliminary evaluations should be made to determine optimal dosage levels of Newcastle virus in challenges. Vaccination practices should be standardized among trials. In a research context, chickens known to be free of Newcastle antibodies should be inoculated with SRBC, and HI and ELISA tests performed to determine possible cross reactivity. Finally, the effects of Newcastle immunization should be tested in chickens which receive NDV alone and in combination with SRBC primary immunization and/or SRBC booster to further explore the correlations between these two antigens.

SUMMARY

Antibody response to Newcastle vaccine and correlations between Newcastle virus and sheep red blood cells (SRBC) were studied in cockerels from lines selected divergently for titers to SRBC. High antibody (HA) line chickens had higher SRBC titers but lower titers to Newcastle-B1 than those from the low antibody (LA) line, regardless of whether antigens were given together or alone. For birds receiving both antigens, within line correlations were positive and significant in line HA and negative but not significant in line LA. Rank correlations among families for Newcastle and SRBC titers were not significant in either line.

Table 8. SRBC titers of chickens receiving SRBC antigen only, by line in Trial 1

Item	Line		Sig.
	HA	LA	
No. chicks	23	13	
Produced SRBC antibody			
No.	21	10	
%	91	77	NS
Means ± s.e. for SRBC titers			
All	3.35 ± .52	1.38 ± .24	*
Responders[1]	3.67 ± .52	1.80 ± .13	*

*P ≤ .05; NS not significant.

[1]Analysis based on only those chicks which produced SRBC antibody.

Table 9. HI titers of chickens receiving Newcastle-B1 (NDV) antigen only, by line in Trial 1

Item	Line HA	Line LA	Sig.
No. chicks	39	17	
Produced NDV antibody			
No.	31	10	
%	79	59	NS
Means ± s.e. for HI titers			
All	3.18 ± .27	2.65 ± .57	NS
Responders[1]	4.00 ± .10	4.50 ± .27	*

*P ≤ .05; NS not significant.

[1]Analysis based on only those chicks which produced NDV antibody.

Table 10. SRBC and MER antibody titers of chickens receiving SRBC antigen only, by line in Trial 2

Item	Line: HA	LA	Sig.
No. chicks	60	52	
Produced SRBC antibody			
No.	47	30	
%	78	58	**
Produced MER antibody			
No.	19	1	
%	32	2	**
Means ± s.e. all chicks			
SRBC	3.03 ± .30	1.56 ± .25	**
MER	0.82 ± .25	0.04 ± .04	**
Means ± s.e. of those that produced SRBC antibody			
SRBC	3.87 ± .28	2.70 ± .30	**
MER	1.04 ± .21	0.07 ± .07	**

*P ≤ .05; **P ≤ .01.

Table 11. SRBC and HI titers of chickens receiving both SRBC and Newcastle-B1 (NDV) antigens, by line in Trial 2

Item	Line HA	LA	Sig.
No. chicks	47	50	
Produced NDV antibody			
No.	47	50	
Produced SRBC antibody			
No.	40	21	
%	85	42	**
Means ± s.e. SRBC			
All	2.87 ± .27	1.10 ± .22	**
Responders[1]	3.38 ± .24	2.62 ± .27	P = .056
Means ± s.e. HI			
All	4.28 ± .14	4.76 ± .12	*
Responders[1]	4.33 ± .16	4.62 ± .19	NS

*P ≤ .05; **P ≤ .01; NS not significant.

[1]Analysis based on only those chicks which produced SRBC antibody.

EXPERIMENT III

PRELIMINARY STUDY ON RELATIONSHIPS BETWEEN SHEEP RED BLOOD CELL, 2-MERCAPTOETHANOL, AND NEWCASTLE-B1 ANTIGENS

INTRODUCTION

Variation in antibody response to sheep red blood cells (SRBC) by chickens is influenced by numerous factors including age, dose, stress, vaccination history, and genetic stock (Subba Rao and Glick, 1977; Van der Zijpp, 1982; 1983b; Van der Zijpp et al., 1982, Ubosi et al., 1985a, 1985b; Experiment 2). The kinetics of primary and secondary immune responses to SRBC antigens also differ. Ubosi et al. (1985b) showed that lines selected divergently for total antibody response to SRBC differed in response to primary immunization with SRBC but not in response to a booster. In an attempt to further evaluate differences between lines for response to Newcastle disease virus (NDV) and relationships between NDV and SRBC titers in the context of removing line differences for response to SRBC, the following preliminary experiment was conducted.

MATERIALS AND METHODS

All chicks used in this experiment were from lines selected for high (HA) and low (LA) production of antibody to sheep red blood cells (Siegel and Gross, 1980). At hatching, 70 HA and 108 LA chicks were wingbanded and vaccinated for Marek's disease. Chicks were reared with lines and sexes intermingled in floor pens. Food and water was provided _ad libitum_.

Chicks were administered .1 ml of .25% suspension of sheep red blood cells (SRBC) in physiological saline at 79 days of age. Five days later blood was taken for determination of total SRBC and 2-mercaptoethanol resistant (MER) antibodies. SRBC antibody titers were determined by microtitration methods (Wegmann and Smithies, 1966) and titers were expressed as $\log_2$ of the inverse of the highest dilution showing agglutination. Relative levels of MER antibodies were determined by the method of Delhanty and Solomon (1966) and were expressed in the same manner as total SRBC titers. At 94 days of age, each bird was given one drop intranasally of a 10^{-6} dilution of Newcastle-B1 virus. A booster of SRBC at the same dosage as previously was given at 105 days of age. Three days later birds were bled for determination of total and MER antibody to SRBC. Antibody to Newcastle virus was determined by hemagglutination inhibition (HI) titers (Beard, 1975), and expressed as $\log_2$ of the inverse of the highest dilution showing inhibition of agglutination.

Chi-square values were calculated to compare lines for frequency of individuals which did and did not produce SRBC, MER and/or HI titers. Analyses of variance were conducted within treatments to determine if line differences existed in SRBC, MER, and HI titers. Analyses were made for all individuals and for only those who produced the respective antibody. Within line correlations between these titers were calculated.

RESULTS AND DISCUSSION

All birds produced antibody to SRBC and NDV, while the number of chicks producing MER titers was significantly greater for line HA than LA. Highly significant differences (HA > LA) were found for mean SRBC and MER titers to primary and booster dosages, as well as for HI titer (Table 12), regardless of whether all chicks were included in the analysis or if the analysis was restricted to those which produced MER titers to primary immunization. When the analysis was restricted to only those individuals which produced MER titers to the booster immunization, differences between lines were highly significant for all assays except for the MER booster titers.

Line differences for SRBC titers to the booster were in contradiction with the findings of Ubosi *et al*. (1985b), who found no significant differences between lines in response to SRBC booster in two trials. In a third trial, however, they observed significantly higher SRBC and MER titers to both primary and booster immunizations with SRBC by line HA than LA chickens.

The observation of HI titers in line HA was consistent with the findings from the third generation birds where challenge with Newcastle-B1 virus resulted in lower mortality and higher HI titers in line HA than in line LA. The mean HI titers reported here were, however, inconsistent with those from Experiment 2, where the HI titers for line LA exceeded those of HA in both trials. HI titers are known to vary with vaccination program and SRBC inoculation (Van der Zijpp et al., 1982), and since different temporal patterns for SRBC response exist between primary and booster immunizations (Van der Zijpp et al., 1983; Ubosi et al., 1985b), it is not unreasonable to assume that HI titers may be affected differentially when given with primary or booster inoculations with SRBC.

All correlations of primary and booster SRBC titers with MER and HI titers were positive and significant in line HA (Table 13). The same general pattern was noted in line LA except for correlations between SRBC titers and MER of the primary immunization. MER titers were also positively correlated to HI titers in both lines, with significance occurring between booster MER titers and HI but not with primary MER titers. This pattern might be expected since HI titers were measured at the same time as the booster responses. The correlations between SRBC and MER were in accordance with those of Ubosi et al. (1985b). While the correlations between SRBC and HI titers obtained here agreed with those reported by Frankena (1980), they should be interpreted with caution as a range of values from .53 to -.54 were reported by Van der Zijpp (1983a).

This preliminary experiment suggests that responses between primary and secondary immunizations may vary with regard to the primary antigen and its relationship with other antigens. Such is not surprising since the relationship between primary and secondary immunizations involve differential responses of IgG and IgM (Barrett, 1983). The complexities of these relationships, however, are rapidly becoming feasible for experimentation through recent technological breakthroughs in the development of monoclonal antibody procedures and the advent of the use of ELISA tests in poultry.

SUMMARY

A preliminary experiment was conducted to explore the relationships between antibody responses to sheep erythrocytes (SRBC), 2-mercaptoethanol resistant (MER) antibodies to SRBC, and Newcastle-B1 vaccine. Chickens used in this experiment were from lines selected for high (HA) and low (LA) antibody response to sheep erythrocytes. Chicks from line HA had higher antibody titers to Newcastle vaccine, and higher SRBC and MER titers to primary and booster inoculations with SRBC antigen than did those from line LA. Within line correlations between titers to Newcastle virus and sheep red blood cells were positive and significant in both lines.

Table 12. SRBC and MER titers, primary and secondary, and HI titers for chickens receiving both SRBC and NDV by line

Item		Line HA	Line LA	Sig.
No. chicks		70	108	
Produced MER antibody				
Primary	No.	50	14	
	%	71	13	**
Booster	No.	44	9	
	%	63	8	**
Means ± s.e. for all chicks				
Primary	SRBC	7.21 ± .19	4.06 ± .15	**
	MER	1.81 ± .17	0.20 ± .05	**
Booster	SRBC	5.46 ± .13	3.21 ± .11	**
	MER	1.77 ± .13	0.31 ± .05	**
	HI	5.57 ± .12	3.99 ± .08	**
Means ± s.e. for responders to MER primary				
Primary	SRBC	7.62 ± .23	4.14 ± .39	**
	MER	2.54 ± .13	1.57 ± .14	**
Booster	SRBC	5.56 ± .14	3.71 ± .34	**
	MER	1.56 ± .15	0.50 ± .25	**
	HI	5.64 ± .17	4.21 ± .24	**
Means ± s.e. for responders to MER booster				
Primary	SRBC	7.66 ± .21	5.56 ± .29	**
	MER	2.34 ± .19	0.67 ± .29	**
Booster	SRBC	5.77 ± .17	4.33 ± .33	**
	MER	2.02 ± .11	1.56 ± .24	NS
	HI	5.80 ± .13	4.67 ± .24	**

$^{*}P \leq .05$; $^{**}P \leq .01$; NS not significant.

Table 13. Phenotypic correlations between titers to SRBC and NDV antigens in HA (above diagonal) and LA (below diagonal) line chickens which received both antigens

		Primary		Booster		
		SRBC	MER	SRBC	MER	HI
Primary	SRBC		.50**	.26**	.31**	.25*
	MER	.03		.26*	.48**	-.16
Booster	SRBC	.37**	.16		.45**	.66**
	MER	.27**	.25**	.30**		.33**
	HI	.48**	.14	.80**	.23*	

*P ≤ .05; ** P ≤ .01.

SUMMARY AND CONCLUSIONS

The experiments reported in this thesis were conducted to examine the role of genetic variation and blood allosystems in resistance to cecal coccidiosis and to Newcastle disease (NDV). Chickens used in these experiments were from lines selected for high (HA) and low (LA) antibody response to sheep erythrocytes (SRBC). Previous studies had shown that these lines differed in resistance to several infectious agents and allelic frequencies at various blood allosystem loci.

Similar results were obtained from natural and controlled exposures to <u>Eimeria</u> <u>tenella</u>, with chicks from line HA being more resistant than those from line LA. In addition, an allele at locus I exhibited additive genetic effects on resistance to <u>E</u>. <u>tenella</u>. Alleles at loci C and D had no notable effects on resistance. Severity of exposure and background genomes affected the phenotypic expression of genetic resistance.

Data where NDV antigen was administered intranasally and SRBC antigen was given intravenously showed that line HA chickens had higher SRBC and and lower NDV titers than did line LA chicks. Within-line correlations between titers in groups of chickens that received both antigens were significant in line HA but not LA. Rank correlations among sire families, where progeny were subdivided so that they received either NDV or SRBC antigen, were not significant. The high frequency of individuals having positive titers to NDV in uninoculated birds indicated that low level infections may play a role in both experimental and field inoculations with NDV.

In a preliminary experiment to further study the relationships between titers to SRBC and NDV, NDV antigen was administered at the time of a booster dose of SRBC antigen. Line HA chicks had higher titers to both NDV and SRBC (primary and booster) than did those from line LA. Product moment correlations between SRBC and NDV titers were positive and significant in each line. Differences in results between this experiment and the previous one indicate that different mechanisms may operate when NDV is given with primary or with booster inoculations of SRBC antigen.

The results of the experiments conducted as part of this thesis show that breeding for resistance to E. tenella is feasible in chickens. More complicated, under existing knowledge, will be development of populations varying in resistance to Newcastle disease.

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ALLOANTIGEN SYSTEMS AND RESISTANCE TO *EIMERIA TENELLA* AND NEWCASTLE-B1 IN CHICKENS SELECTED FOR RESPONSE TO SHEEP ERYTHROCYTES

by

Alison Martin

(ABSTRACT)

Chickens from lines selected for high (HA) and low (LA) antibody response to sheep red blood cell (SRBC) antigen were used to study the role of genetic factors involved in resistance to *Eimeria tenella* and Newcastle disease virus (NDV). Routes of administration were intravenous for SRBC antigen and intranasal for NDV. Chicks were exposed to *E. tenella* either through a natural challenge via the litter or a controlled oral administration. Differences between lines were observed in resistance to cecal coccidiosis, with line HA chicks being more resistant than those from line LA. Results were similar for both natural and controlled exposures. Differences in resistance to *E. tenella* were found among alleles for the I allosystem with degree of additivity from the I^4 allele on resistance depending on the background genome.

Chicks from line HA exhibited higher antibody titers to SRBC and lower titers to NDV than did those from line LA. This pattern was the same regardless of whether antigens were given together or alone. Correlations within lines for birds which received both antigens were positive and

significant in line HA and not different from zero in line LA. When NDV was given at the time birds received a booster dose of SRBC, antibody titers for NDV and for primary and booster SRBC were higher in line HA than line LA. Correlations between all titers were positive and significant in both lines. The data suggest that relationships between titers for SRBC and NDV are influenced by both genetic and nongenetic factors.