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Alloantigen System *L* Affects Antibody Responses[1]

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Abstract: Alloantigen system *L* is a polymorphic protein expressed on the surface of chicken erythrocytes and possibly certain leukocyte subpopulations. Previous studies demonstrated that the *L* system affects Rous sarcoma outcome and phagocytic function. The present experiments examined the L system influence on antibody responses to two antigens: SRBC (T-dependent) and *Brucella abortus* (BA, partially T-independent) in three *B* complex genotypes. The parental stock were 50% Modified Wisconsin Line 3 x White Leghorn Line NIU 4 and 50% Inbred Line 6-15.5. Pedigree matings of 4 $B^2B^5L^1L^2$ sires to 5 $B^2B^5L^1L^2$ dams per sire produced three hatches (n = 183) for the antibody response to SRBC and two hatches (n = 198) to study antibody response to BA. At 4 and 11 weeks of age the experimental birds were injected intravenously with 1 mL of 2.5% SRBC or 0.1 mL of 10% BA. Total and mercaptoethanol (ME)-resistant primary and secondary titers were analyzed by least squares ANOVA. Alloantigen *L* had a significant effect on total primary antibody titer to SRBC in a B^5B^5 background ($p < 0.004$) and on total ($p < 0.011$) and ME-resistant ($p < 0.017$) secondary titer to SRBC in the B^2B^5 genotype. Total ($p < 0.004$) and ME-resistant ($p < 0.005$) secondary titers to BA in B^2B^5 chickens were significantly affected by alloantigen *L*. The data indicate that the alloantigen *L* locus or genes in the immediate chromosomal vicinity affect antibody responses to SRBC and BA.

Key words: Erythrocyte alloantigen, major histocompatibility (*B*) complex, *L* alloantigen, *Brucella abortus*

Introduction

Alloantigen *L* is one of thirteen described systems expressed on the surface of chicken erythrocytes. Chicken leukocytes also appear to express this alloantigen on their surface (W. E. Briles, Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115, unpublished data) but these data were not conclusive. The *L* system is polymorphic with L^1 and L^2 alleles (Gilmour, 1959; Briles, 1962). Both the L^1 and L^2 alleles encode antigens of approximately 135kD (Ameiss and Briles, 2000). Previous research has shown that allele combinations of the *L* system influence a range of immune responses including the fate of Rous sarcoma virus (RSV)-induced tumors (LePage *et al.*, 2000), phagocytosis (Qureshi *et al.*, 2000) and response to *Eimeria tenella* (Taylor and Briles, 2000).

LePage *et al.* (2000) studied segregating combinations of genes encoding eight alloantigen systems for their effects on Rous sarcomas in both B^2B^5 and B^5B^5 MHC backgrounds. Among these alloantigens, *L* alleles were the only system that affected Rous sarcoma growth. The L^1L^1 genotype was associated with a stronger anti-sarcoma response, manifested as lower tumor score, TPI, and mortality in the B^2B^5 background compared with the L^1L^2 genotype. In the B^5B^5 background, the L^1L^1 genotype had lower mortality than the L^1L^2 genotype. Medarova *et al.* (2002) examined the effect of fully segregating combinations of *L* system alleles on Rous sarcomas in three *B* complex genotypes: B^2B^2, B^2B^5 and B^5B^5. That study found an *L* effect only in a B^5B^5 genetic background. The L^1L^2 genotype had lower tumor scores, TPI, and mortality than the L^1L^1 and L^2L^2 genotypes. The strong anti-sarcoma response in the B^2B^2 and B^2B^5 genotypes masked L effects. The differences between these two studies in the observed *L* alloantigen effects were attributed to the different genetic backgrounds of the experimental chickens and different RSV doses.

Qureshi *et al.* (2000) analyzed the effect of alloantigen systems on phagocytosis following *in vitro* incubation of monocyte monolayers with viable *E. coli*. The *L* system had a significant effect on monocyte function that was independent of the *B* system. In 4 wk old birds, the L^1L^1 genotype had a significantly higher percentage of phagocytic monocytes than the L^1L^2 genotype. Other experiments found no L alloantigen effect on macrophage nitrite or IL-6 production (M. A. Qureshi, Department of Poultry Science, N. C. State University, Raleigh, NC 27695, unpublished data).

Taylor and Briles (2000) examined alloantigen systems for their effects on resistance and susceptibility to *E. tenella* in B^2B^2 and B^2B^5 backgrounds. Only the *L* system affected the response among the eight alloantigen systems studied. The effect on cecal lesion scores was evident in a B^2B^2 background but not in a B^2B^5 background. $B^2B^2L^1L^1$ chickens had higher lesion scores than $B^2B^2L^1L^2$ and $B^2B^2L^2L^2$ chickens.

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Certain immune related selection experiments demonstrated changes in *L* allele frequencies. Selection for size of the bursa of Fabricius altered the frequency of *L* alleles, as well as antibody responses to SRBC (Scott *et al.*, 1988). IgG levels were also altered by the bursa size selection. Chickens selected for differential primary antibody response to SRBC (Dunnington *et al.*, 1984; Martin *et al.*, 1990) became fixed for the L^2 type.

Based on these studies, we hypothesized that the *L* system modulates, directly or indirectly, the potential for producing an effective antibody response to foreign antigens. We examined the *L* alloantigen effect on primary and secondary responses to SRBC and BA in the identical line of chickens used by our laboratory to study the *L* system's role in Rous sarcoma outcome (Medarova *et al.*, 2002). These chickens have two *L* alloantigen alleles fully segregating with three *B* complex haplotypes such that the *L* effects can be analyzed in the context of different *B* genotypes. This mating also serves to minimize the influence of other background genes.

Materials and Methods

Stock: Chickens for this study were derived from several inbred lines. Congenic Line 6.15-5 (Dix and Taylor, 1996) was produced by crossing USDA-ADOL inbred Line 15_1 (B^5B^5) and Line 6_1 (B^2B^2) followed by ten backcross generations to Line 6_1. Heterozygous B^2B^5 chickens from the tenth backcross generation were mated *inter se* to produce Line 6.15-5 B^5B^5 birds that have 99.9% of the Line 6_1 genetic background. Modified Wisconsin Line 3 is an experimental population derived from Wisconsin inbred Line 3 Ancona (95% inbred) [McGibbon, 1978], produced by introducing selected alloantigen system alleles from White Leghorns and backcrossing two or more generations to Line 3. White Leghorn Line NIU 4 was derived over 20 generations from *inter se* matings of crosses between four commercial parent stocks and selecting for equal frequencies of alloantigens segregating at 9 alloantigen loci.

Modified Wisconsin Line 3 x Line NIU 4 sires ($B^2B^2L^1L^2$) were crossed to inbred Line 6.15-5 dams ($B^5B^5L^1L^1$) to produce the parental stock consisting of 50% inbred Line 6.15-5 with the $B^2B^5L^1L^2$ genotype. Pedigree matings of 4 sires to 5 dams per sire produced experimental progeny segregating for all possible combinations of *B* and *L* haplotypes. Three hatches having 183 chickens were used for SRBC injection and two hatches having 198 progeny were injected with BA. The birds were hatched at the University of New Hampshire Poultry Research Farm and were wing-banded for identification. Vaccinations against Marek's disease and Newcastle-bronchitis were administered at hatch and 10 d, respectively. The birds were housed in heated brooder batteries with water and food freely available. Six-week-old chicks were transferred to isolation cages for the remainder of the experiment.

Alloantigen typing: The chickens were typed for *B* and *L* systems in agglutination assays utilizing antisera specific for the haplotypes of the parental stocks (Briles and Briles, 1982) as described (LePage *et al.*, 2000). After the chicks reached 3 wk of age, 0.5 ml blood was drawn from the wing vein into cold sodium citrate anticoagulant solution (68 µM sodium citrate / 72 µM sodium chloride). Samples were shipped overnight with ice packs to Northern Illinois University. Fifty µl of a 2% suspension of washed red blood cells was dispensed into tubes containing 100µl of antiserum specific for the *B* or *L* system haplotypes of interest. Following a 2-hr room temperature incubation, the reaction mixtures were transferred to 3 °C for an overnight incubation. The following day, cells were resuspended, incubated for 1 hr at room temperature, and scored visually for agglutination.

Antigen Inoculation: At 4 weeks of age, the experimental birds were injected intravenously with 1 mL of a 2.5% solution of SRBC[2] in Alsever's solution. A different group of segregating chicks was injected with 0.1 mL of a 10% solution of *Brucella abortus* standard tube test antigen[3]. Blood samples were taken 7 d post-injection. Serum from SRBC and BA inoculated birds was separated and stored for the titer assay. Each procedure was repeated in the same birds at 11 weeks of age to assay for secondary total and ME-resistant antibody titers to the specific antigens.

Antibody Titration: Standard microtiter techniques were used to assay for levels of serum antibodies to SRBC. Total anti-SRBC antibody levels were assayed according to the method described by Wegman and Smithies (1966). Total BA antibody was assayed according to the procedure described by McCorkle and Glick (1980). Mercaptoethanol (ME)-resistant antibody (IgG) levels to SRBC and BA were determined using the method of Yamamoto and Glick (1982). Titer was expressed as the $\log_2$ of the reciprocal of the highest dilution having a visible agglutination.

Statistical Analysis: The goal was to assess *L* system effects in each of three *B* genotypes. Total and ME-resistant antibody titers for the primary and secondary response were analyzed by least squares ANOVA with hatch, sire, dams within sires, and *L* genotype as main effects. Significant means were separated by Fisher's protected LSD.

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Results

The total and ME-resistant antibody to SRBC from primary as well as secondary responses were analyzed for an *L* alloantigen effect within each *B* genotype. A significant *L* effect ($p < 0.004$) on the total primary antibody response to SRBC within a B^5B^5 background ($n = 38$) was found. In these birds, the L^1L^2 genotype was associated with the lowest total primary titer (5.38 ± 0.34). That titer was significantly lower than the titers of the L^2L^2 (6.67 ± 0.56) and L^1L^1 (6.6 ± 0.47) birds (Fig. 1). Analysis also revealed a significant *L* influence on both total ($p < 0.011$) and ME-resistant ($p < 0.017$) secondary antibody response to SRBC within a B^2B^5 genotypic background. Among the B^2B^5 genotype ($n = 99$), the total secondary antibody titer to SRBC in the L^1L^1 birds was significantly lower (7.6 ± 0.35) than both L^1L^2 (7.98 ± 0.31) and L^2L^2 (7.94 ± 0.27) birds (Fig. 2). The B^2B^5 L^1L^1 birds also had a significantly lower ME-resistant secondary antibody titer to SRBC (6.5 ± 0.37) than either the B^2B^5 L^1L^2 (7.0 ± 0.25) or $B^2B^5L^2L^2$ (7.0 ± 0.21) genotypes (Fig. 2).

Alloantigen *L* did not affect primary antibody responses against Brucella abortus (BA). The secondary total and ME-resistant anti-BA responses revealed significant *L* system effects in one *B* genotype. The *L* genotype significantly affected total ($p < 0.004$) and ME-resistant ($p < 0.005$) secondary titer to BA within a B^2B^5 genotypic background ($n = 100$). The L^1L^1 genotype had the highest total secondary titer to BA (7.58 ± 0.12) that differed significantly from L^2L^2 birds (6.6 ± 0.07) but not L^1L^2 (7.13 ± 0.05) birds (Fig. 3). The ME-resistant secondary titer to BA was also significantly higher in the B^2B^5 L^1L^1 genotype (6.96 ± 0.12) than in the B^2B^5 L^2L^2 (6.1 ± 0.08) but not the B^2B^5 L^1L^2 (6.77 ± 0.06) genotype (Fig. 3).

Discussion

The segregating combinations of the B^2 and B^5 haplotypes did not affect the antibody response to either antigen. Dix and Taylor (1996) found *B* complex effects on total and ME-resistant primary antibody against SRBC and BA in which congenic Line 6-15.5 (B^5B^5) had significantly higher antibody than congenic Line 6-6.2 (B^2B^2). On the other hand, Bacon *et al.* (1987) showed no difference in anti-SRBC or BA antibody titers between congenic lines 15.6-2 (B^2B^2) and 15.15-5 (B^5B^5) having the Line $15I_5$ genetic background. Three points address these divergent results. First, the two congenic series have different genetic backgrounds ($15I_5$ vs. 6_1). Second, the 15.*B* lines had undergone five backcross generations at the time of testing, suggesting that other genes may have affected the responses. Finally, Bacon *et al.* (1987) injected the SRBC and BA antigens simultaneously, which may have modulated the response. The lack of a *B* complex effect in the current study suggests that background genes may have affected the antibody response.

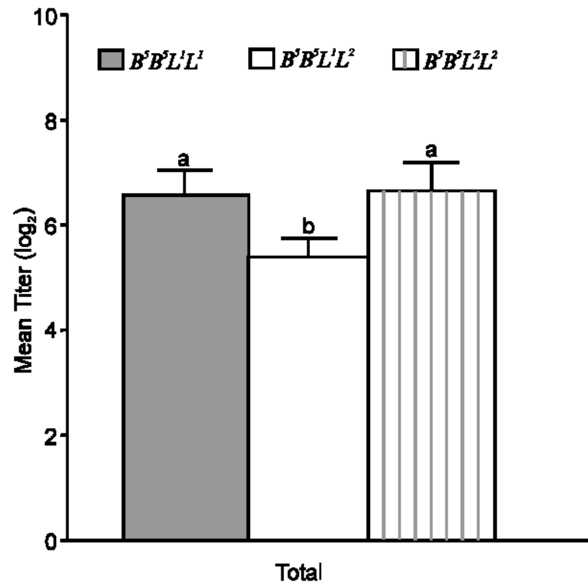

Fig. 1: Mean primary antibody titers to SRBC for L^1L^1 (n = 10), L^1L^2 (n = 16), and L^2L^2 (n = 12) genotypes within a B^5B^5 background. Bars having no common letter differ significantly (P < 0.05).

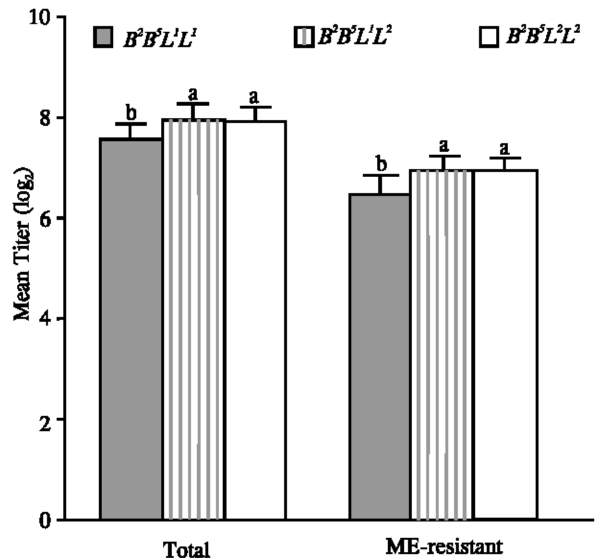

Fig. 2: Mean total and ME-resistant secondary antibody titers to SRBC for L^1L^1 (n = 20), L^1L^2 (n = 48), and L^2L^2 (n = 31) genotypes within a B^2B^5 background. Bars within an antibody type having no common letter differ significantly (P < 0.05).

The only significant *L* effect on primary antibody response was against SRBC within a B^5B^5 background. The L^1L^2 genotype had significantly lower total primary antibody compared with either of the homozygous *L* genotypes. Qureshi *et al.* (2000) found that the L^1L^1 genotype had

significantly greater monocyte phagocytosis of *E. coli* than the L^1L^2 genotype, an effect that was independent of the *B* system. The L^2L^2 genotype was not produced in that study. Lower SRBC phagocytosis by the L^1L^2 genotype would be plausible and consistent with the lower antibody level of that genotype in the B^5B^5 MHC birds.

Genotype B^2B^5 exhibited L alloantigen effects on the secondary anti-SRBC response. The L^1L^1 birds had significantly lower total and ME-resistant antibodies than did L^1L^2 or L^2L^2 birds. The secondary responses to a T-dependent antigen produce higher affinity antibody compared with primary responses. Memory B cells have higher affinity antigen receptors. Memory T cells respond to lower doses of antigen that suggests greater receptor efficiency. Increased numbers of reactive cells as well as altered expression of cell-surface molecules and cytokines are another secondary response characteristic (Ullman *et al.*, 1989). The *L* system may affect T cells, B cells, or both populations within the B^2B^5 genotype, thereby influencing the secondary response. Alloantigen L does not influence macrophage nitrite or IL-6 production (M. A. Qureshi, Department of Poultry Science, N. C. State University, Raleigh, NC 27695, unpublished data). Altered production of other cytokines by macrophages or other cell types has not been examined.

With respect to the BA antibody response, we found no *L* system effect on primary antibody response but a significant effect on secondary antibody response in the B^2B^5 genotypic background. Polyclonal activation by BA may negate any response differences due to alloantigen *L*. The lack of observable primary effect could also be a reflection of the partial T-cell independence of the immune response to BA. Efficient immune responses to BA do involve both CD4+ and CD8+ T cells to some degree, as well as efficient isotype switching in the course of a humoral response (Golding *et al.*, 2001). The *L* system effect on secondary response to BA is only evident in a B^2B^5 background as found in the secondary response to SRBC. This may result from similar effects on cell populations responding to BA in the secondary response.

Scott *et al.* (1988) found changes in *L* alloantigen allele frequencies in chickens divergently selected for bursa of Fabricius size. The L^2 allele had an average frequency of 0.02 and 0.54 in the Large Bursa Line (LBL) and the Small Bursa Line (SBL), respectively. The LJD base line was fixed for L^1, which occurred after selection was initiated. The SBL had significantly higher primary and secondary antibody against SRBC compared with the LBL. In contrast, serum IgG was lower in the SBL than in the LBL (Landreth and Glick, 1973; Yamamoto and Glick, 1982). No causal relationship between *L* allele frequency changes and these immune effects was demonstrated but the results were not entirely due to the *B* complex, suggesting non-MHC gene effects including *L* (Landreth and Glick, 1973; Yamamoto and Glick, 1982). Rees and Nordskog (1980) found significant differences in basal serum IgG levels among inbred lines but no differences between IgG levels according to *B* haplotypes segregating within these lines providing further support for

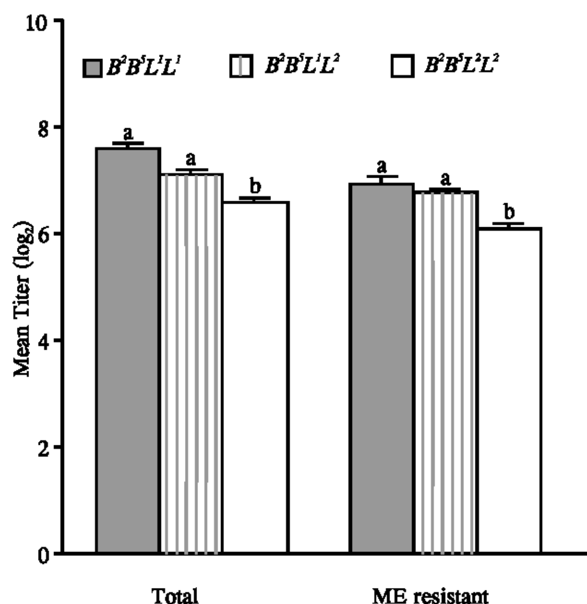

Fig. 3: Mean total and ME-resistant secondary antibody titers to BA for L^1L^1 (n = 24), L^1L^2 (n = 47), and L^2L^2 (n=29) genotypes within a B^2B^5 background. Bars within an antibody type having no common letter differ significantly ($P < 0.05$).

non-MHC gene effects on IgG.

L allele frequencies were shifted through selection for primary antibody response to SRBC (Dunnington *et al.*, 1984; Martin *et al.*, 1990). In the High Antibody (HA) Line and the Low Antibody (LA) Line, the L^2 allele was fixed despite segregation for both L^1 and L^2 alleles in the Cornell randombred stock, which formed the base population. A founder effect could not be excluded as contributing to the *L* frequency changes. Selection for high or low antibody response shifted *B* haplotype frequencies such that the HA and LA Lines are at or near fixation for B^{21} and B^{13}, respectively.

Different *L* genotypes have distinct effects on primary versus secondary titer depending on the *B* genotype context. Interactions between cells bearing *L* and *B* antigens or between the antigens themselves may occur. An alternative explanation is that the *L* alloantigen may have a pleiotropic effect on T or B cells involved in the antibody response. Precedents exist for *L* alleles having dissimilar effects in different *B* genotypes. Rous sarcoma outcome was affected by *L* genotype in two separate investigations. The L^1L^1 genotype had an enhanced response to Rous sarcomas compared to the L^1L^2 genotype in B^2B^5 birds (LePage *et al.*, 2000). Medarova *et al.* (2002) examined the *L* influence on Rous sarcomas in fully segregating combinations of two *B* haplotypes and two *L* alleles. The L^1L^2 genotype was associated with an enhanced response compared to the L^1L^1 and L^2L^2 genotypes in B^5B^5 birds. These two studies employed matings with different background genes and used different virus doses. Certain parallels exist between alloantigen L and CD22, an antigen influencing mouse and human antibody

responses. CD22 is a 130 to 140 kD MW antigen expressed during B cell functional maturation. This molecule modulates the signaling threshold for B cell activation and proliferation following B cell receptor engagement (Tedder *et al.*, 1997). B cell CD22 interacts with other B cells, monocytes, and T cells (Stamenkovic *et al.*, 1991). Only two serologically-defined alleles that differ in polypetide coding sequences have been described. These two alleles vary among inbred strains of mice (Lajaunias *et al.*, 1999). The L system has a 135 KD molecular weight and possesses two alleles as does CD22. Adding the possible L expression on white blood cells, the similarities between the two systems raise intriguing possibilities that merit further investigation.

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