Alloantigen System *L* Affects the Outcome of Rous Sarcomas[1]

ZDRAVKA MEDAROVA,* W. ELWOOD BRILES,† AND ROBERT L. TAYLOR, JR.[2,*]

**Department of Animal and Nutritional Sciences, University of New Hampshire, Durham, New Hampshire 03824; and †Department of Biological Sciences, Northern Illinois University, DeKalb, Illinois 60115*

This study was designed to examine the alloantigen system *L* effects on Rous sarcomas in three *B* complex genotypes. The parental stock was 50% Modified Wisconsin Line 3 × White Leghorn Line NIU 4 and 50% inbred Line 6.15-5. Pedigree matings of two B^2B^5 L^1L^2 sires to five B^2B^5 L^1L^2 dams per sire produced experimental chicks segregating for *B* and *L* genotypes. Chicks were inoculated with 20 pock-forming units (pfu) of Rous sarcoma virus (RSV) at 6 weeks of age. Tumors were scored six times over 10 weeks postinoculation after which the tumor scores were used to assign a tumor profile index (TPI) to each chicken. Tumor growth over time and TPI were evaluated by repeated-measures analysis of variance and analysis of variance, respectively. Six trials were conducted with a total of 151 chickens. The major histocompatibility (*B*) complex affected the responses as the B^2B^2 and B^2B^5 genotypes had significantly lower tumor growth over time and TPI than the B^5B^5 genotype. Separate analyses revealed no significant *L* system effect in B^2B^2 or B^2B^5 backgrounds. However, *L* genotype significantly affected ($P < 0.05$) both tumor growth over time and TPI in B^5B^5 chickens. B^5B^5 L^1L^2 birds had TPI significantly lower than B^5B^5 L^1L^1 chickens but not B^5B^5 L^2L^2. Mortality was lower in the B^5B^5 L^1L^2 birds than in B^5B^5 L^2L^2 chickens. The *L* system, or one closely linked, affects the growth and ultimate outcome of Rous sarcomas. The response may depend upon the genetic background as well as MHC type.

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Genetic variation in antigenic determinants among members of a species produces alloantigens. Numerous alloantigens have been described based on immunogenetic analysis of antisera resulting from exchange of blood between individual animals. Chicken erythrocyte alloantigen systems *A, B, C, D, E, H, I, J, K, L, N, P,* and *R* (1) have been identified by serological reactions or by other methods. The *B* blood group was one of the earliest alloantigen systems described (2). Schierman and Nordskog (3) subsequently found that the *B* blood group was associated with skin homograft tolerance and thus established that the *B* system was the major histocompatibility complex (MHC) in the chicken. The *B* complex has a pivotal role in immune responsiveness and the outcome of pathogenic challenges (4, 5).

Gilmour (6) and Briles (7) independently discovered the *L* alloantigen system and established that two haplotypes, L^1 and L^2, segregate. The *L* locus segregates independently from nine other erythrocyte alloantigen systems (1), but has not been assigned to a linkage group. Alloantigen typing showed that the *L* system did not segregate (8) in a reference population established for molecular mapping. In addition to the described *L* haplotype associations with responses to Rous sarcomas virus (RSV)-induced tumors, other investigations have revealed *L* allele frequency alterations following divergent selection for bursa size (9) and fertility changes among different *L* genotypes (10). Taylor and Briles (11) examined the effect of eight non-MHC alloantigen systems on resistance and susceptibility to *Eimeria tenella* in both B^2B^2 and B^2B^5 backgrounds. The authors found an association only between the *L* system and cecal lesions. $B^2B^2L^1L^1$ chickens had higher lesion scores than $B^2B^2L^1L^2$ and $B^2B^2L^2L^2$ chickens. No significant *L* genotype effect was observed in a B^2B^5 background.

Rous sarcoma is a connective tissue tumor caused by the RSV, an oncogenic RNA virus. Tumors develop after injection of the virus into susceptible chickens. The tumors may regress or progress depending on the level of antitumor immune response produced by the MHC (12, 13). Variation in RSV tumor outcome among identical MHC genotypes from crosses of inbred lines differing at the MHC (12, 14–

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[2] To whom requests for reprints should be addressed at Department of Animal and Nutritional Sciences, University of New Hampshire, Kendall Hall, Durham, NH 03824. E-mail: bob.taylor@unh.edu

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17), among different inbred lines identical at the *B* complex (18–20), or among crosses of noninbred lines (21, 22) implicated a role for non-MHC genes. For example, non-MHC T lymphocyte alloantigens, *Ly-4* and *Th-1*, and the B lymphocyte alloantigen *Bu-1* interacted to alter the response against RSV tumors in crosses of B^2B^2 inbred lines (19, 20).

Two previous studies found *L* alloantigen effects on the response to Rous sarcomas. Collins (23) studied the effect of alloantigen systems *C, D, E, I,* and *L* on tumor outcome in the F_2 generation of inbred lines $6_3 \times 100$ that all had the B^2B^2 genotype. The *C, D, E,* and *I* systems did not influence tumor outcome. On the other hand, the *L* genotype significantly affected tumor growth in females, but not in males. LePage *et al.* (24) examined the effect of alloantigen systems *A, C, D, E, H, I, L,* and *P* on Rous sarcoma outcome in two *B* complex genotypes: B^5B^5, a tumor progressor, and B^2B^5, a moderate progressor. Alloantigen systems *A, C, D, E, H, I,* and *P* had no significant effect on tumor fate. The *L* genotype correlated with a differential tumor outcome. In the B^2B^5 genotypic background, the L^1L^1 chickens had lower tumor size, TPI, and mortality than the L^1L^2 chickens. Mortality was lower in L^1L^1 birds compared with L^1L^2 birds in the B^5B^5 background.

These earlier experiments indicate significant *L* alloantigen modulation of immune responses without complete *B* and *L* system segregation. The objective of this study is to further investigate *L* system effects on RSV-induced tumors. We used crosses producing progeny fully segregating for both the *B* complex and the *L* alloantigen. This structure allows examination of the *L* system effects in *B* genotypes that vary widely in their RSV-induced tumor outcome.

Materials and Methods

Stock. Chickens for this study were derived from several lines. Line 6.15-*5* is a congenic line (25) that was produced by crossing USDA-ADOL inbred Line 15_1 (B^5B^5) to USDA-ADOL inbred Line 6_1 (B^2B^2). After 10 backcross generations, heterozygous B^2B^5 chickens 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) (26), originally homozygous for genes of all alloantigen loci except the *B* system. The modification consisted of introducing alloantigens for selected systems 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 nine alloantigen loci.

Line 6.15-*5* (B^5B^5 L^1L^1) dams were mated to B^2B^2 L^1L^2 sires from a line cross between modified Wisconsin Line 3 Ancona × White Leghorn line NIU 4 sires (B^2B^2 L^1L^2), as described by LePage *et al.* (24). Chickens from this mating that had the B^2B^5 L^1L^2 genotype contained 50% of the Line 6.15-*5* genome and were used as parents to produce the experimental progeny. Pedigree matings of two B^2B^5 L^1L^2 sires to five B^2B^5 L^1L^2 dams per sire produced six hatches having one hundred fifty-one chicks segregating for all possible combinations of *B* and *L* genotypes. 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 days, respectively. The chicks 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 (27). When chicks reached 3 weeks of age, 0.5 ml of blood was drawn from the wing vein to 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 microliters of a 2% suspension of washed red blood cells was dispensed into tubes containing 100μl of antiserum specific for the *B* and *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 and scored visually for agglutination after a 1-hr incubation at room temperature.

RSV Challenge and Tumor Evaluation. At six weeks of age, the birds were inoculated in the right wingweb with 20 pfu of the Bryan high-titer strain of RSV (RAV-1), subgroup A. Two weeks following RSV challenge, tumors were scored for size using the following scale: 0, no palpable tumor; 1, small tumor up to 0.5 cm in diameter; 2, tumor >0.5 up to 1.2 cm in diameter; 3, tumor >1.2 cm up to one-half of wingweb area; 4, tumor > one-half of wingweb area, but < entire wingweb area; 5, tumor filling the entire wingweb; 6, massive tumor extended beyond wingweb; and 7, death during the experiment (12). Tumor size was also scored at weeks 3, 4, 6, 8, and 10 postinoculation for a total of six tumor size scores over the 10-week experimental period. The six tumor size scores were then used to assign a TPI to each bird as an indicator of the tumor growth pattern. The TPI values were those of Collins *et al.* (28) where 1 = complete regression by 70 days postinoculation, or a decreasing slope, or complete regression by 56 days followed by recurrence; 2 = general upward trend, or plateau; slight regression after 56 days; 3 = terminal tumor after 42 days postinoculation; 4 = terminal tumor between 29 and 42 days postinoculation; and 5 = terminal tumor by 28 days postinoculation.

Statistical Analysis. Tumor scores were analyzed by repeated measures analysis of variance (ANOVA) with hatch, sex, sire, dams within sire, time, *B* genotype, *L* genotype, and a *B* × *L* interaction as main effects. A large effect of *B* type led to separate analyses for each *B* genotype having hatch, sire, dams within sire, time, and *L* type as

main effects. The TPI values were rank transformed and analyzed by ANOVA as described by Conover and Iman (29) with the same independent variables except time as in the repeated measures ANOVA. Significant differences between alloantigen system genotypes were determined using Fisher's protected LSD. Mortality rates were evaluated using chi-square analysis.

Results

Alloantigen system genotypes for *B* and *L* segregated independently of each other in the 151 progeny. Repeated measures analysis of tumor score revealed a significant ($P = 0.0001$) *B* genotype effect on tumor growth. The significant ($P = 0.0001$) interaction between time and *B* genotype indicated that tumor growth differed over time for the B^2B^2, B^2B^5, and B^5B^5 genotypes (Fig. 1A). Analysis of the TPI values demonstrated a highly significant *B* genotype effect ($P = 0.0001$). The highest mean TPI, found in the B^5B^5 genotype ($n = 31$; TPI = 3.35 ± 0.18), was significantly greater than the TPI of B^2B^2 birds ($n = 46$; TPI = 1.43 ± 0.16) and B^2B^5 birds ($n = 74$; TPI = 1.36 ± 0.10; Fig. 1B). The *B* genotype significantly affected ($P = 0.0001$) mortality rates of 13.1%, 10.8%, and 77.4% for the B^2B^2, B^2B^5, and B^5B^5 genotypes, respectively (Table I, Analysis 1). No differences between males and females were detected.

The overwhelming *B* genotype effect on tumor outcome led to separate analyses of *L* genotype effects on tumor score, TPI, and mortality within each specific *B* genotype. Therefore, L^1L^1, L^1L^2, and L^2L^2 birds were analyzed within the B^2B^2, B^2B^5, and B^5B^5 MHC types. No significant effect of *L* genotype on tumor score over time, TPI, or mortality was evident in either the B^2B^2 or B^2B^5 (data not shown) MHC genotype backgrounds. Both the B^2B^2 and B^2B^5 genotypes from this mating exhibited strong regression of RSV-induced tumors.

The *L* genotype, however, exerted a significant influence on tumor score, TPI, and mortality in the B^5B^5 genotypic background. Repeated measures analysis of tumor score indicated a significant change in tumor size over time ($P = 0.0001$), as well as a significant *L* genotype × time interaction ($P = 0.00017$). The overall pattern of tumor growth was an increase in tumor size 2, 3, and 4 weeks postinoculation, followed by a lower rate of tumor size increase in B^5B^5 L^1L^2 ($n = 14$) compared with either the B^5B^5 L^1L^1 ($n = 5$) or B^5B^5 L^2L^2 ($n = 12$) genotypes (Fig. 2A). Tumor size at 10 weeks postinoculation was diminished in the L^1L^2 genotype (5.43 ± 0.6) compared with either the L^1L^1 (7.00 ± 0.0) or L^2L^2 (6.83 ± 0.2) genotypes.

ANOVA of the TPI values also found a significant *L* genotype effect on tumor outcome ($P = 0.023$) in B^5B^5 chickens. The L^1L^2 genotype had TPI of 2.93 ± 0.3, which was significantly lower than the TPI values of L^1L^1 (4.00 ± 0.3), but not L^2L^2 (3.58 ± 0.3) chickens (Fig. 2B). Furthermore, chi-square analysis of mortality differences revealed a significant *L* effect ($P = 0.046$). The L^1L^2 genotype had the lowest mortality rate (57.1%), whereas the L^2L^2 and L^1L^1 genotypes had mortality rates of 91.7% and 100%, respectively (Table I, Analysis 2).

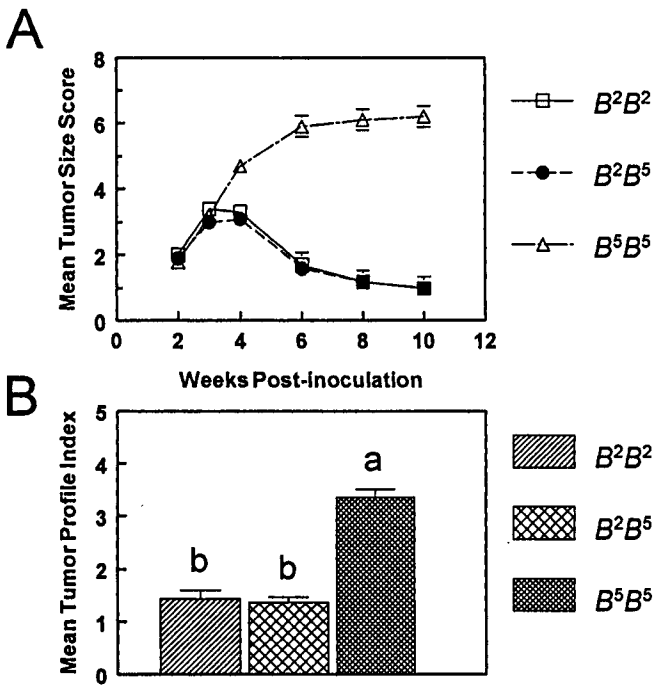

Figure 1. Effect of *B* genotype on tumor size scores over a 10-week experimental period (A) and TPI in B^2B^2 ($n = 46$), B^2B^5 ($n = 74$), and B^5B^5 ($n = 31$) chicks inoculated with 20 pfu of Rous sarcoma virus (B). Tumor growth for the three *B* genotypes differs significantly ($P = 0.0001$) over time. Bars having no common letter differ significantly ($P < 0.05$).

Discussion

Segregating combinations of two *B* and two *L* haplotypes produced nine different genotype combinations in the experimental progeny. The results are consistent with the differential B^2B^2 and B^5B^5 genotype effects on retroviral oncogene tumors reported previously. B^2B^2 chickens regress RSV tumors or v-*src* DNA tumors, whereas B^5B^5 hosts progress these tumors (12, 30). The same divergent *B* complex responses are evident in tumor metastasis (31, 32) as well as in immunity to a second inoculation of RSV or v-*src* DNA (32, 33). Other B complex haplotypes also differ in their responses to RSV tumors or v-*src* DNA tumors (34–36). Heterozygous B^2B^5 chickens usually have less tumor regression than B^2B^2 chickens (12, 31). The current study assessed different mating types compared with former experiments, therefore the profound similarity in B^2B^2 and B^2B^5 genotypes tumor growth may be due to non-MHC genes.

The *L* alloantigen genotypes were analyzed within each *B* genotype to eliminate the possibility that the powerful *B* complex effect would overcome any *L* system effects. No significant *L* effect was evident in either the B^2B^2 or B^2B^5 genotypic background, as both genotypes regressed tumors. A significant *L* influence, however, was evident in the B^5B^5 background. This result suggests that the robust tumor re-

Table I. Contingency Table Chi-Square Analyses of Mortality Due to Rous Sarcomas among Different Genotypes in Modified Wisconsin Line 3 x 6.15-5 White Leghorn Chickens

Analysis	Genotype	Alive		Dead		X^2	P
		n	%	*n*	%		
1	B^2B^2	40	86.9	6	13.1	56.63	0.0001
	B^2B^5	66	89.2	8	10.8		
	B^5B^5	7	22.6	24	77.4		
2	$B^5B^5L^1L^1$	0	0	5	100	6.14	0.046
	$B^5B^5L^1L^2$	6	42.9	8	57.1		
	$B^5B^5L^2L^2$	1	8.3	11	91.7		

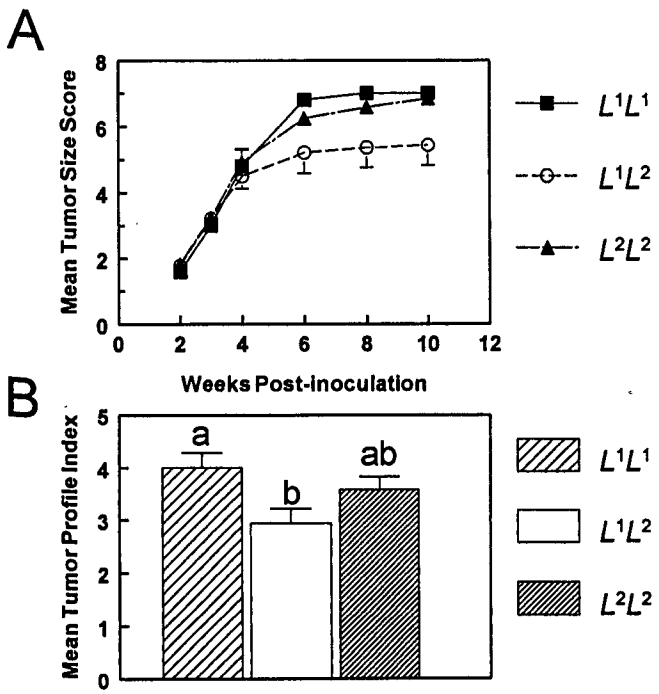

Figure 2. Effect of *L* alloantigen genotype on tumor size scores over a 10-week experimental period (A) and TPI in B^6B^6 L^1L^1 ($n = 5$), B^6B^6 L^1L^2 ($n = 14$), and B^6B^6 L^2L^2 ($n = 12$) chicks inoculated with 20 pfu of Rous sarcoma virus (B). Tumor growth for the three *L* genotypes differs significantly ($P = 0.00017$) over time. Bars having no common letter differ significantly ($P < 0.05$).

gression found in B^2B^2 and B^2B^5 birds masks the weak *L* effect, which becomes obvious only in the B^5B^5 progressor background. Based upon tumor growth over time, TPI, and mortality, the L^1L^2 genotype mitigated the progressive effect of the B^5B^5 genotype and did so significantly compared with the L^1L^1 genotype.

Two prior studies examined the *L* alloantigen system influence on Rous sarcomas. Among the F_2 generation of B^2B^2 inbred lines 6_3 x 100, tumor fate was affected significantly by *L* genotype (23). Females of the L^1L^1 genotype had significantly lower TPI than the L^2L^2 genotype. No *L* genotype effect was found in males. LePage *et al.* (24) tested B^2B^5 and B^5B^5 progeny that segregated for at least two alleles of eight non-*B* alloantigen systems. Significant *L* alloantigen effects on Rous sarcomas were found in both *B* complex backgrounds. The L^1L^1 genotype was associated with lower tumor scores, TPI, and mortality than the L^1L^2 genotype in a B^2B^5 background. Lower mortality was found in B^5B^5 L^1L^1 chickens than in B^5B^5 L^1L^2 chickens.

The apparent disagreement of the present data with previous studies may be attributed to several factors. First, each study used a mating type with a different genetic background. Collins (23) used progeny from the cross of two inbred lines, LePage *et al.* (24) used progeny that were 50% Modified Wisconsin Line 3 and 50% White Leghorns, and the current study used progeny that consisted of 50% inbred line 6-15.5 and 50% Modified Wisconsin Line 3 x NIU 4 White Leghorns. Second, LePage *et al.* (24) used a higher virus dose (30 pfu) than this experiment (20 pfu). Virus dose may influence tumor growth in progressive genotypes (37), such as B^5B^5. Third, the current study is the only one that utilizes full segregation of *B* and *L* haplotypes. Effects of background genes, other than *B* and *L*, cannot be completely excluded by the results.

Growth and subsequent division of tumor cells as well as cellular recruitment via viral-replication genes (30) affect RSV tumor growth. T cells are principally responsible for RSV tumor regression (17, 19, 38, 39). Cross-reactions between tumor antigens and certain MHC haplotypes may impinge on antigen recognition. The B_5 antigen cross-reacted to one or more RSV tumor antigens because Rous sarcoma progression increased in B^2B^2 chickens previously made tolerant to the B_5 antigen from progressor chickens compared with untreated B^2B^2 controls (40, 41). Tumor regressor Line CB $B^{12}B^{12}$ chickens progressed a transplantable v-*src*-induced tumor after they were made tolerant to Line CC B^4B^4 or CB.R1, (B-F^{12} B-G^4). This result supported a cross-reaction between the B_4 antigen and a v-*src* tumor antigen (36).

The L^1L^2 genotype advantage in B^5B^5 tumor outcome indicates complementation. The host response to a tumor is a complex reaction to a multitude of antigens (42). Particular heterozygous combinations of MHC molecules may facilitate tumor or viral antigen recognition (43). Compared with the homozygotes, a heterozygote may complement through either improved recognition efficiency of the same number of antigens or increased recognition of additional antigens. The L^1L^2 genotype or closely linked genes, in B^5B^5 chickens of the current study, may have enhanced the immune response against RSV tumors by interacting with an effector molecule, facilitating viral or tumor antigen in-

teraction with effector molecules or partially overcoming the B_5 antigen-associated tolerance to tumor antigens. These effects might be accomplished through increased T cell activation, *B* complex antigen expression, or both.

Plachy (44) described MHC and non-MHC gene complementation in crosses of the Prague congenic inbred lines, CB ($B^{12}B^{12}$), CB.I (B^7B^7), and inbred line IA (B^7B^7). Chickens of the $B^{12}B^7$ genotype from either CB × CB.I or CB × IA matings had more regressing tumors than either $B^{12}B^{12}$ or B^7B^7 chickens, indicating complementation between the B^{12} and B^7 haplotypes. In addition, greater tumor regression was evident in the $B^{12}B^7$ genotype CB × IA chickens than in the same genotype from the CB × CB.I mating, denoting a non-MHC gene effect that was complementary. Matings of Line CC (B^4B^4) with CB.I and IA revealed similar MHC and non-MHC complementation that was independent of age. B^4B^7 chickens from the CC × IA mating had more tumor regression than CC × CB.I cross (44).

Other non-MHC genes have demonstrated complementary effects on the outcome of Rous sarcomas. Gilmour *et al.* (19) studied progeny derived from inbred line crosses identical for the MHC (B^2B^2) but segregating for *Ly-4* and *Th-1* non-MHC T cell-surface antigen genes. A homozygous/heterozygous interaction was found in that *Ly-4*^{a}*Ly-4*^{a}*/Th-1*^{a}*Th-1*^{b} and *Ly-4*^{a}*Ly-4*^{b}*/Th-1*^{a}*Th-1*^{a} genotypes had increased RSV tumor regression. The interaction between the *a* and *b* haplotypes occurred when the other locus was the *aa* genotype. Another example of complementation occurred between non-MHC B and T cell alloantigens, *Bu-1* and *Ly-4*. Progeny from a different cross of B^2B^2 inbred lines had greater RSV tumor regression due to complementation between the *Ly-4*^{a} and *Bu-1*^{b} alleles or the *Ly-4*^{b} and *Bu-1*^{a} alleles (20).

Genes other than the MHC can affect the RSV tumor progressive B^5B^5 genotype. Collins *et al.* (22) examined Rous sarcoma metastasis in B^5B^5 hosts from two populations: (Line 6_1 × Line 15_1) F_5 White Leghorn cross and (Line 6_1 × Line 15_1) F_5 Leghorn × Line UNH 105 New Hampshire F_2. The incidence of tumor metastasis was significantly lower in the B^5B^5 White Leghorn × UNH 105 cross than in the B^5B^5 White Leghorn population, suggesting a possible non-MHC background effect on metastasis. Another study found that B^5B^5 birds having alloantigen haplotypes D^{1+} or I^{8+} had significantly lower TPI than those with D^{1-} or I^{8-} (24). Mortality in B^5B^5 chickens was lowered by the L^1L^1 genotype compared with the L^1L^2 genotype (24). However, that study used chickens with a different genetic background and used two *B* genotypes with three *L* genotypes compared with the current research that has full segregation of *B* and *L* genotypes.

The present experiments revealed that the *B* complex and the *L* alloantigen system significantly affected tumor growth over time, TPI, and mortality due to RSV-induced tumors. This work also adds unique information regarding *L* alloantigen effects on the immune response to Rous sarcomas in the context of B^2 and B^5 haplotype segregation. Complementation by the L^1L^2 genotype in the B^5B^5 birds supports the conclusion that the *L* alloantigen system, or some closely linked gene(s), has significant effects on the fate of Rous sarcomas. Further research should examine possible interactions between the *L* system, different *B* haplotypes, and other non-MHC genes.

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1. Dietert MF, Taylor RL Jr, Dietert RR. Avian blood groups. Poult Sci Rev **4**:87–105, 1992.
2. Briles WE, McGibbon WH, Irwin MR. On multiple alleles effecting cellular antigens in the chicken. Genetics **35**:633–652, 1950.
3. Schierman LW, Nordskog AW. Relationship of blood type to histocompatibility in chickens. Science **134**:1008–1009, 1961.
4. Dietert RR, Taylor RL Jr, Dietert MF. Biological functions of the chicken major histocompatibility complex. Crit Rev Poult Biol **3**:111–129, 1991.
5. Kaufman J, Salomonsen J. The "minimal essential Mhc" revisited: both peptide-binding and cell surface expression of MHC molecules are polymorphisms selected by pathogens in chickens. Hereditas **127**:67–73, 1997.
6. Gilmour DG. Segregation of genes determining red cell antigens at high levels of inbreeding in chickens. Genetics **44**:14–33, 1959.
7. Briles WE. Additional blood group systems in the chicken. Ann NY Acad Sci **97**:173–183, 1962.
8. Crittenden LB, Provencher L, Santangelo L, Levin I, Abplanalp H, Briles RW, Briles WE, Dodgson JB. Characterization of a red jungle fowl by white Leghorn backcross reference population for molecular mapping of the chicken genome. Poult Sci **72**:334–348, 1993.
9. Scott TR, Oduho GW, Glick B, Hagan F, Briles WE, Yamamoto Y. Erythrocyte alloantigen diversity and some immunological effects of the B system in related New Hampshire strains. Poult Sci **67**:1210–1217, 1988.
10. DeSilva PLG. Heterozygocity at red cell antigen locus *L* and fertility in chickens. Genetics **51**:41–48, 1965.
11. Taylor RL Jr, Briles WE. Non-MHC alloantigen effects on resistance and susceptibility to *Eimeria tenella*. Poultry Sci **79**(Suppl. 1):39, 2000.
12. Collins WM, Briles WE, Zsigray RM, Dunlop WR, Corbett AC, Clark KK, Marks JL, McGrail TP. The B locus (MHC) in the chicken: association with the fate of RSV-induced tumors. Immunogenetics **5**:333–343, 1977.
13. Schierman LW, Watanabe DH, McBride RA. Genetic control of Rous sarcoma regression in chickens: linkage with the major histocompatibility complex. Immunogenetics **5**:325–332, 1977.
14. Brown DW, Collins WM, Zsigray RM, Briles WE. A non-MHC genetic influence on response to Rous sarcoma virus-induced tumors in chickens. Avian Dis **28**:884–899, 1984.
15. Plachy J, Hala K, Benda V. Regression of tumors induced by virus in Rous sarcoma different inbred lines of chickens. Folia Biol (Praha) **25**:335–336, 1979.
16. Plachy J. Hierarchy of the *B* (MHC) haplotypes controlling resistance to Rous sarcomas in a model of inbred lines of chickens. Folia Biol (Praha) **30**:412–425, 1984.
17. Plachy J, Benda V, Karakoz I, Spatenkova Z. Different importance of

primary lymphoid organs for Rous sarcoma regression in inbred lines of chickens. Folia Biol (Praha) **30:**177–188, 1984.
18. Collins WM, Heinzelmann EW, Corbett AC, Zsigray RM, Dunlop WR. Rous sarcoma regression in seven highly inbred lines of white leghorns. Poult Sci **59:**1172–1177, 1980.
19. Gilmour DG, Collins WM, Fredricksen TL, Auclair B, Clark KK, Urban WE Jr. Influence of non-MHC T lymphocyte alloantigens on regression of Rous sarcomas in the chicken. Immunogenetics **17:**43–54, 1983.
20. Gilmour DG, Collins WM, Fredricksen TL, Urban WE. Genetic interaction between non-MHC T- and B-cell alloantigens in response to Rous sarcomas in chickens. Immunogenetics **23:**1–6, 1986.
21. Collins WM, Briles WE, Corbett AC, Clark KK, Zsigray RM, Dunlop WR. B locus (MHC) effect upon regression of RSV-induced tumors in noninbred chickens. Immunogenetics **9:**97–100, 1979.
22. Collins WM, Brown DW, Ward PH, Dunlop WR, Briles WE. MHC and non-MHC genetic influences on Rous sarcoma metastasis in chickens. Immunogenetics **22:**315–321, 1985.
23. Collins WM. Genetic influences on the fate of RSV-induced tumors in chickens. In: Poultry Breeders of America, Eds. Proceedings of the 28th National Breeder's Roundtable. Kansas City, MO: Poultry Breeders of America, pp1–14, 1979.
24. LePage KT, Briles WE, Kopti F, Taylor RL Jr. Nonmajor histocompatibility complex alloantigen effects on the fate of Rous sarcomas. Poult Sci **79:**343–348, 2000.
25. Dix MC, Taylor RL Jr. Differential antibody responses in 6.*B* major histocompatibility (*B*) complex congenic chickens. Poult Sci **75:**203–207, 1996.
26. McGibbon WH. The genetics of green spotting on shanks of Ancona chickens. J Hered **69:**97–100, 1978.
27. Briles WE, Briles RW. Identification of haplotypes of the chicken major histocompatibility complex (*B*). Immunogenetics **15:**449–459, 1982.
28. Collins WM, Zervas NP, Urban WE Jr, Briles WE, Aeed PA. Response of *B* complex haplotypes B^{22}, B^{24}, and B^{26} to Rous sarcomas. Poult Sci **64:**2017–2019, 1985.
29. Conover WJ, Iman RL. Rank transformations as a bridge between parametric and nonparametric statistics. Am Statistician **35:**124–129, 1981.
30. Taylor RL Jr, Ewert DL, England JM, Halpern MS. Major histocompatibility (*B*) complex control of the growth pattern of v-*src* DNA-induced primary tumors. Virology **191:**477–479, 1992.
31. Collins WM, Dunlop WR, Zsigray RM, Briles RW, Fite RW. Metastasis of Rous sarcoma tumors in chickens is influenced by the major histocompatibility (*B*) complex and sex. Poult Sci **65:**1642–1648, 1986.
32. Taylor RL Jr, England JM, Kopen GC, Christou AA, Halpern MS. Major histocompatibility (*B*) complex control of the formation of v-*src*-induced metastases. Virology **205:**569–573, 1994.
33. Guyre PM, Zsigray RM, Collins WM, Dunlop WR. Major histocompatibility complex (*B*): effect on the response of chickens to a second challenge with Rous sarcoma virus. Poult Sci **61:**829–834, 1982.
34. Plachy J, Karakoz I, Benda V. Concomitant immunity leads to retardation or acceleration of the growth of Rous sarcomas in genetically resistant or susceptible inbred lines of chickens. Folia Biol (Praha) **32:**334–348, 1986.
35. Svoboda J, Plachy J, Hejnar J, Karakoz, I, Guntaka RV, Geryk, J. Tumor induction by the LTR, v-*src*, LTR DNA in four *B* (MHC) congenic lines of chickens. Immunogenetics **35:**309–315, 1992.
36. Plachy J, Hala K, Hejnar J, Geryk J, Svoboda J. *src*-specific immunity in inbred chickens bearing v-*src* DNA- and RSV-induced tumors. Immunogenetics **40:**257–265, 1994.
37. Vincent SC, Taylor RL Jr. Virus dilution affects the anti-Rous sarcoma response of progressor but not regressor major histocompatibility (*B*) complex genotypes. Poult Sci **67:**1491–1496, 1988.
38. Cotter PF, Collins WM, Dunlop WR, Corbett AC. The influence of thymectomy on Rous sarcoma regression. Avian Dis **20:**75–78, 1976.
39. Powell PC, Hala K, Bock G, Dietrich H, Wick G. Immune mechanisms in Rous sarcoma regression. In: Weber WT, Ewert DL, Eds. Avian Immunology. New York: Alan Liss, pp207–218, 1987.
40. Heinzelmann EW, Zsigray RM, Collins WM. Cross-reactivity between RSV-induced tumour antigen and B_5 MHC alloantigen in the chicken. Immunogenetics **13:**29–37, 1981.
41. Heinzelmann EW, Zsigray RM, Collins WM. Increased growth of RSV-induced tumours in chickens partially tolerant to MHC alloantigens. Immunogenetics **12:**275–284, 1981.
42. Brown DW, Collins WM, Ward PH, Briles WE. Complementation of major histocompatibility haplotypes in regression of Rous sarcoma virus-induced tumors in noninbred chickens. Poult Sci **61:**409–413, 1982.
43. Lukacs NW, Briles WE, Briles RW, Taylor RL Jr. Response of major histocompatibility (*B*) complex haplotypes B^{22}, B^{26} and B^{30} to Rous sarcomas. Poult Sci **68:**233–237, 1989.
44. Plachy J. Age-dependent complementation of the *B* (MHC) alleles and a non-B allele controlling resistance to progression of Rous sarcomas, and genetic independence of resistance to induction of tumours in the model of inbred lines of chickens. Folia Biol (Praha) **31:**284–296, 1985.