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Genetic Interaction between Non-MHC T- and B-Cell Alloantigens in Response to Rous Sarcomas in Chickens

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Abstract. Chickens of Regional Poultry Research Laboratory (RPRL) inbred line 6_3 regress sarcomas induced by Bryan high-titer Rous sarcoma virus to a greater extent than chickens of line RPRL 100, although these lines are identical for the major histocompatibility *B* complex. They differ, however, at three independent autosomal loci: *Ly-4* and *Th-1* determine the surface alloantigens of partly overlapping subsets of T lymphocytes, and *Bu-1* determines a surface alloantigen of B lymphocytes. The association of genotypes at these loci with quantitative variation in their ability to regress Rous sarcomas was tested in segregating F_4 generation progeny derived from crosses of lines 100 and 6_3. The *Ly-4* and *Bu-1* genotypes showed association with Rous sarcoma regression, but the *Th-1* genotype did not. Chickens of the $Ly\text{-}4^a/Ly\text{-}4^a$, $Bu\text{-}1^b/Bu\text{-}1^b$ and $Ly\text{-}4^b/Ly\text{-}4^b$, $Bu\text{-}1^a/Bu\text{-}1^a$ genotypes had a significantly higher regressor ability than the other two double homozygous genotypes. These results indicate that higher regression is associated with (1) interaction between the *Ly-4* and *Bu-1* loci, and (2) complementation between either the line 6 $Ly\text{-}4^a$ allele and the line 100 $Bu\text{-}1^b$ allele, or the line 100 $Ly\text{-}4^b$ allele and the line 6 $Bu\text{-}1^a$ allele.

Introduction

In chickens susceptible to the initiation of tumors by Rous sarcoma virus (RSV), progressive growth or regression of sarcomas is strongly affected by the genetic variation of the host, as was first clearly demonstrated by Gyles and co-workers (1968). This conclusion was confirmed in experiments on selection for a high incidence of regression within closed populations (Gyles and Brown 1971, Carte et al. 1972). In both cases, response to selection was relatively rapid, suggesting the important influence of relatively few loci. It was subsequently shown (Collins et al. 1977, Schierman et al. 1977) that the *Ea-B* blood group locus [major histocompatibility complex (MHC) (Pazderka et al. 1975a, b, Pink et al. 1977)], or a gene (or genes) linked to it, markedly influenced Rous sarcoma regression. Schierman and co-workers (1977) proposed that strong regression was determined by a single autosomal dominant gene linked to the MHC. This gene was later shown to be associated with the *B-F/B-L* region of the complex rather than the *B-G* region from studies of MHC-recombinant chickens (Collins and Briles 1980, Plachy and Benda 1981). Thus, genes coding for lymphocyte antigens (B-F/B-L) rather than erythrocyte antigens (B-G) were involved.

The influence of non-MHC genes on regression can be inferred from two sets of observations. First, there was considerable residual variation in regression within the *B*-genotypic regressor groups (B^2/B^2 and B^2/B^5) in the F_2 generation of crosses between two MHC-different Regional Poultry Research Laboratory (RPRL) lines 6_1 (or 6_3) and 15_1 (Collins et al. 1977, Bacon et al. 1981). Second, there were consistent differences in regression between MHC-identical inbred lines 6_1 or 6_3 on the one hand, and 7_2 or 100 on the other (Marks et al. 1979, Collins et al. 1980). In both these situations, a previously proposed hypothesis (Gilmour et al. 1976, Fredericksen et al. 1977) involving a role for non-MHC-coded lymphocyte surface antigens in determining host responses to viral oncogenesis has been tested. The T-cell antigens involved are coded by two independent loci, *Ly-4* and *Th-1* (Gilmour et al. 1976, Fredericksen et al. 1977, Fredericksen and Gilmour 1981), and the B-cell antigens are coded by another independent locus, *Bu-1* (Gilmour et al. 1976). Th-1 antigens are expressed on most thymus cells and to a

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lesser extent on peripheral T cells, while Ly-4 antigens are expressed on a few thymus cells and most peripheral T cells (Fredericksen and Gilmour 1981). Because of this distribution, and because some T cells carry both antigens, Th-1 and Ly-4 may represent markers for early and late stages of T-cell maturation, respectively.

Lines 6_3 and 15_1 differ at the two non-MHC T-cell loci, as well as at the MHC, but in tests of B^2/B^5 F_6 progeny, no significant association was found between the four homozygous genotypes at these loci and the residual variation in Rous sarcoma regression found in this *B*-genotypic class (Bacon et al. 1985). In contrast, tests of F_4 and F_5 generations derived from crosses of *B* MHC-identical lines 6_3 and 7_2 demonstrated a clear association between genotypes at the T-cell antigen loci and variation in regression (Gilmour et al. 1983). In the F_4 generation, tests of the four homozygous genotypes showed that the double homozygote for the *a* alleles derived from the stronger regressor parental line 6_3 (*Ly-4^a/Ly-4^a*, *Th-1^a/Th-1^a*, symbolized by *aa/aa*) possessed a significantly higher regressor ability than the other three genotypes. Our evidence for homozygous interaction between the two loci was confirmed and extended in tests of all nine possible genotypes in the F_5 generation. The results showed that homozygous/heterozygous interaction also occurred, since the *aa/ab* and *ab/aa* genotypes for Ly-4 and Th-1, as well as genotype *aa/aa*, had significantly higher regression than any of the other six genotypes (*b* being in each case the allele derived from the lesser regressor line 7_2). The overall conclusion was that at each locus *a* was dominant to *b* for higher regression, but that interaction occurred such that dominance occurred only within the *aa* genotype at the other locus.

Two sets of association tests have been carried out in generations derived from a further cross of inbred lines, RPRL $100 \times 6_3$. Line 100 is partly congenic with 7_2, but differs from it in that it expresses the *Tva^s* gene for dominant susceptibility to subgroup A leukosis sarcoma viruses (Crittenden et al. 1967). It was derived from crosses of lines $6 \times 7_2$, followed by four backcrosses to line 7_2 (Stone 1975, Fredericksen and Gilmour 1985). In the F_3 generation from $100 \times 6_3$, evidence was obtained for association of *Bu-1* homozygous genotypes with regression, the *aa* genotype showing higher regression ($P < 0.05$, Bacon et al. 1985). *Ly-4* genotypes could not be determined, and *Th-1* genotypes were expected to occur in a 1:2:1 ratio in each of the *Bu-1* genotypic groups. We now report RSV regression tests on eight homozygous genotypic classes at the three lymphocyte antigen loci *Ly-4*, *Th-1*, and *Bu-1* in the F_4 generation of the $100 \times 6_3$ cross, which show that interaction between Ly-4 and Bu-1, but not Th-1, determines variation in regressor ability.

Materials and Methods

Chickens. The highly inbred line RPRL 6_3 (inbreeding coefficient >0.99) (Stone 1975) was developed at the USDA Regional Poultry Research Laboratory, East Lansing, Michigan, by selection for resistance to the "Leukosis complex" followed by inbreeding (Gilmour et al. 1983). This line is homozygous for B^2 (Pazderka et al. 1975b), *Ly-4^a* (Fredericksen et al. 1977), and *Th-1^a* and *Bu-1^a* (Gilmour et al. 1976). Line 100 was derived from crosses of lines RPRL $6 \times 7_2$, followed by four backcrosses to 7_2 with selection for alleles at the *tva* and *tvb* tumor virus susceptibility loci, and then intercrossing (Stone 1975). It is thus partly congenic with the highly inbred line RPRL 7_2 (inbreeding coefficient >0.99), and like this line is homozygous for B^2, *Ly-4^b*, *Thy-1^b*, and *Bu-1^b*.

The original matings from which our test progeny were derived were carried out at the RPRL between a single male of line 100, which had been shown by progeny testing to be homozygous for dominant susceptibility to subgroup A leukosis sarcoma viruses (*Tva^s/Tva^s*), and six females of line 6_3 known to be similarly homozygous. In the next generation, 5 F_1 males were mated to 3–7 F_1 females each, avoiding full-sib matings, and over 400 F_2 chicks were produced. Of these, about 100 males and 160 females were tested for their phenotype, and hence genotype, for lymphocyte antigens determined by *Ly-4*, *Th-1*, and *Bu-1* (see below). Matings were then set up at the RPRL which were designed to produce a high proportion of the eight possible triple homozygous genotypes for these three loci, and the F_3 progeny were provided as hatching chicks. All the above matings were arranged and supervised by Mr. John V. Motta and Dr. L. D. Bacon of the RPRL, to whom we extend grateful thanks.

The F_3 chicks were reared on the farm of our collaborator Mr. Howard Stone (Omega Chicks, Haslett, Michigan) and were typed for lymphocyte antigens at ages varying from 2–5 months. Matings by artificial insemination between like triple homozygotes, avoiding full and half-sibs, were then set up to provide F_4 progeny known by pedigree to be triple homozygotes. All the selected F_3 breeders were retyped before matings began. The F_4 progeny were shipped to The University of New Hampshire as four fortnightly collections of hatching eggs. Upon hatching, the chicks were vaccinated against Marek's disease, and against Newcastle disease and infectious bronchitis at 10 days of age.

Lymphocyte alloantigen genotypes. Phenotypes (and hence genotypes) of potential parents in the F_2 and F_3 generations were determined from citrated blood samples obtained by Dr. L. D. Bacon and Mr. Howard Stone. The lymphocyte-specific alloantisera for Ly-4, Th-1, and Bu-1 antigens were made using backcross progeny of the inbred lines RPRL 6_3 and 7_2, as previously described (Fredericksen and Gilmour 1981). The lymphocyte antigen tests were performed on lymphocyte suspensions prepared from blood by slow-speed centrifuging at $62 \times g_{max}$ using two-stage indirect immunofluorescence with fluoresceinated goat antichicken Fc (gamma-specific) serum as a second reagent (Fredericksen and Gilmour 1985). The lymphocyte typing was completed within 30 h of blood collection.

Viruses and virus inoculation. A highly purified preparation of Bryan high-titer subgroup A RSV [designated BH RSV(RAV-1), abbreviated RSV-1] was used. The stock virus was kindly provided by Dr. L. B. Crittenden from the RPRL. Chickens were inoculated in the left wing web with 0.1 ml of a 10^{-2} dilution of virus at 6 weeks of age. Dosage was approximately 1280 pock-forming units, based on titration of the virus stock (Hitchner et al. 1975a) by inoculation on the chorioallantoic membranes of susceptible SPAFAS embryos (Hitchner et al. 1975b).

Determination of sarcoma regression. Two weeks after inoculation, each chicken was examined for tumor development in the wing web

once a week for 3 consecutive weeks and every other week thereafter, which gave a total of six readings over a 10-week period. Tumors were scored subjectively for size on a scale which ranged from 0–6, based on the criteria of Collins and co-workers (1977). Using the tumor scores, a tumor profile index (TPI) was determined for each chicken, based initially on the criteria of Collins and co-workers (1977). It was found that the TPI values determined in this way were not normally distributed, but instead were negatively skewed. This situation arose because the present population, which was all B^2/B^2, contained large numbers of regressor individuals, whereas the previous population studied by Collins and co-workers (1977), which was segregating for *B*, had a wide range of variation between regression and progression. We accordingly developed new criteria for TPI values from 1–5 more suited to the variation in regression in our population as follows: 1, complete regression by 4 weeks or earlier, with all or most of the regression taking place between weeks 2 and 3; 2, complete regression by 4 weeks, with all or most of the regression taking place between weeks 3 and 4; 3, complete regression by 6 weeks; 4, complete regression by 8 or 10 weeks, or earlier complete regression followed by recurrence; 5, no complete regression, or death with terminal tumor prior to 10 weeks. On this basis, the distribution of the TPI values was near normal.

Statistical analysis. Least-squares analysis of variance for unequal subclass numbers (Snedecor and Cochran 1967) was used to test TPI values with *Ly-4* genotypes, *Th-1* genotypes, *Bu-1* genotypes, and sex as factorial elements and hatches as block effects. The Statistical Package for the Social Sciences (SPSS) program (Nie et al. 1975) was used first, primarily as a test of the three-way interactions. The least-squares means, standard errors for the main effects, and two-way interactions were then obtained using the Least-Squares Maximum Likelihood General Program of Harvey (1968). Significance was determined at the 0.05 level.

Results

From four hatches, a total of 548 chickens developed Rous sarcomas and were assigned TPI values. The SPSS analysis (not shown) indicated that the *Ly-4* × *Bu-1* interaction was statistically significant, but that the main effects and higher order interactions were not. The least-squares analysis (Table 1) confirmed that the *Ly-4* × *Bu-1* interaction was the only significant source of variation. The least-squares means obtained in this analysis for the four *Ly-4* × *Bu-1* interaction genotypes, together with the numbers of individuals, are given in Table 2. These means reflect the combined influence of the *Ly-4* and *Bu-1* loci on Rous sarcoma regression in the F_4 generation derived from the cross of lines 100 × 6_3. The nature of the interaction is such that the mean TPI values are lowest (regression incidence highest) for the *aa/bb* and *bb/aa* genotypes. These results show that complementation was involved, in that interaction favorable to regression occurred when either the line 6_3 *Ly-4*^{a} and line 100 *Bu-1*^{b} alleles, or the line 6_3 *Ly-4*^{b} and line 100 *Bu-1*^{a} alleles, were present in the homozygous state.

Table 1. Analysis of variance of TPI with *Ly-4* × *Th-1* × *Bu-1* genotypes in $F_4(100 \times 6_3)$

Source	d.f.	Mean square
Hatch	3	12.74
Sex	1	0.01
Ly-4	1	0.16
Th-1	1	0.23
Bu-1	1	0.02
Sex × *Ly*	1	0.28
Sex × *Th*	1	0.18
Sex × *Bu*	1	0.42
Ly × *Th*	1	0.05
Ly × *Bu*	1	11.25*
Th × *Bu*	1	1.71
Residual	534	1.06

* Probability of associated F ≤ 0.05

Table 2. Least-squares mean TPI values (number in group) of *Ly-4* × *Bu-1* genotypes in $F_4(100 \times 6_3)$, based on data used for Table 1

		Bu-1	
		aa	*bb*
Ly-4	*aa*	3.14 (143)	2.84 (88)
	bb	2.79 (164)	3.11 (153)

Probability of associated F with 1 and 534 d.f. ≤ 0.05

Discussion

It is clear that by far the largest influence on the regression of Rous sarcomas is exerted by genotypes at the MHC, or *B* complex. Thus, Collins and co-workers (1977) reported that only 5% of B^2/B^2 individuals in the F_2 generation from crosses of RPRL lines 6_1 and 15_1 (B^2 and B^5, respectively) died with terminal tumors, compared to 93% of B^5/B^5 individuals. Similar large variation was seen in another study on *B* genotypes (Schierman et al. 1977), where only 8% of B^6/B^{13} individuals died with terminal tumors compared to 96% of B^{13}/B^{13} individuals in progeny from backcrosses of line G-B1 (B^{13}/B^{13}) to G-B1 × G-B2 (B^6/B^{13}). The results of Collins and co-workers also showed large variation when expressed as genotypic mean TPI values, namely, 2.94 for B^2/B^2, 3.77 for B^2/B^5, and 4.93 for B^5/B^5. In contrast, the range of variation associated with non-MHC genotypes was smaller in the study of Gilmour and co-workers (1983), being 1.92–2.95 in F_4 ($6_3 \times 7_2$) and 2.33–3.08 in F_5 ($6_3 \times 7_2$).

The possibility that non-MHC genotypes might be associated with further variation in regression was first

suggested by the finding of marked individual variation in TPI values within each of the two regressor genotypic groups (B^2/B^2 and B^2/B^5) in F_2 ($6_1 \times 15_1$) in the study of Collins and co-workers (1977). Similar observations were made by Bacon and co-workers (1981) in F_2 progeny derived from crosses of a different subline, RPRL 6_3, with line 15_1. Consistent differences in sarcoma regression between certain MHC-identical inbred lines (Marks et al. 1979, Collins et al. 1980) also suggested non-MHC genetic effects. These differences were not as large as the MHC-associated differences previously reported, but were quite substantial. For example, the incidence of deaths with progressive tumors was 2% and 10% for the sublines RPRL 6_1 and 6_3, respectively, compared with 55% for line 7_2 and 62% for its partly congenic line 100, with corresponding mean TPIs of 2.0 and 2.9, compared with 3.6 and 4.5 (Collins et al. 1980). On a different background, a substantial effect on regression by a non-MHC locus was inferred by Cutting and co-workers (1981) from progeny of both backcrosses derived from lines G-B1 (B^{13}) and G-B3 (B^{15}), each of which exhibit 100% deaths with progressive tumors following inoculation with B subgroup Schmidt-Ruppin RSV. Although, as expected, almost 100% of the B^{13}/B^{13} or B^{15}/B^{15} backcross progeny exhibited progressive tumor growth, only 50% of B^{13}/B^{15} showed similar progressive growth, and the other 50% regressed their tumors. Cutting and co-workers concluded that resistance to progressive growth in the latter 50% resulted from transcomplementation between a gene within the MHC and a non-MHC gene.

With respect to lines 6_3 (or 6_1) and 7_2, we had earlier postulated that resistance to viral oncogenesis of B or T cells, in which these lines were known to differ, might be associated with surface properties of these cells detectable as alloantigens (Gilmour et al. 1976, Fredericksen et al. 1977). Genotypes at the two T-cell loci, *Ly-4* and *Th-1*, were shown to interact in determining quantitative variation in ability to regress Rous sarcomas in F_4 generation progeny from crosses of lines 6_3 and 7_2 (Gilmour et al. 1983). There was homozygous × homozygous interaction, in that only $Ly\text{-}4^a/Ly\text{-}4^a$, $Th\text{-}1^a/Th\text{-}1^a$ (symbolized *aa/aa*) chickens had significantly higher regressor ability than the other three double homozygotes. In the F_5 generation, all nine possible homozygous and heterozygous genotypes were tested. The previous interaction was confirmed, and heterozygous × homozygous interaction was also demonstrated in that the *aa/ab* and *ab/aa* genotypes, as well as *aa/aa*, showed significantly higher regressor ability than the other six genotypes. This interaction involved dominance, because the *a* allele at each locus was dominant over *b* for higher regression only within the homozygous *aa* genotype at the other locus.

In the present work the *Bu-1* locus, which determines surface antigens on B cells, was included in the association tests in addition to the two T-cell loci, and all of the eight possible homozygous genotypes at the three loci were tested. Although Rous sarcoma regression is largely T-cell mediated (Rubin 1962, Cotter et al. 1976), we postulated that a B-cell antigen locus was also involved, on the basis of our earlier findings on in vitro proliferative responses of peripheral blood leukocytes to concanavalin A and phytohemagglutinin. Although these responses primarily concern T cells (Greaves et al. 1968, Toivanen and Toivanen 1973), they were strongly influenced by three-way interaction between all three non-MHC antigen loci in F_6 generation progeny from the cross of lines 6_3 and 7_2 (Gilmour and Fredericksen 1981). The *aa/aa/aa* triple homozygote for Ly-4, Th-1, and Bu-1, which reconstitutes the genotype of the low responder line 6_3, was by far the lowest responder among segregating F_6 progeny. Our present study on Rous sarcoma regression, using $F_4(100 \times 6_3)$ progeny, provided evidence of Ly-4 × Bu-1 interaction, and not Ly-4 × Th-1 interaction, as observed in earlier studies with F_4 and F_5 generation progeny from the cross of lines 6_3 and 7_2 (Gilmour et al. 1983). Moreover, an interaction favoring regression did not occur as previously described when the *a* alleles from the more regressor line 6_3 were together. Complementation occurred instead, since the *aa/bb* and *bb/aa* genotypes for Ly-4 and Bu-1 had higher regressor ability. The reason for the difference between the two sets of findings is not clear, but it may be related to the partly different genetic background introduced from line 100, rather than from 7_2. Because line 100 was derived from four backcrosses of line 7_2 to $(6 \times 7_2)$, it would be expected to have only about 93.8% of its genes in common with 7_2.

We previously observed that interaction between the non-MHC lymphocyte antigen loci in their association with resistance to viral oncogenesis may involve complementation between alleles from different lines. RPRL lines 6_3 and 15_1 differ for MHC haplotypes and for alleles at the *Ly-4* and *Th-1* loci. In tests of incidence of Marek's disease among highly susceptible B^5/B^5 F_7 progeny from crosses of these two lines, homozygous interaction between $Ly\text{-}4^b$ from line 15_1 and $Th\text{-}1^a$ from line 6_3 was associated with increased resistance. The incidence of Marek's disease was 56.4% for *bb/aa* hosts, compared with 81.9% for chickens of the other three genotypes (Fredericksen et al. 1982). Bacon and co-workers (1985) tested for Rous sarcoma regression among F_6 progeny from a cross of the same two lines. In B^2/B^5 heterozygotes (intermediate regressors) there was no significant association of *Ly-4* or *Th-1* genotypes with regression, but the two complementing genotypes *bb/aa* and *aa/cc* showed a tendency toward increased regression. Those results resemble our present findings.

Complementation between an MHC allele and a non-MHC allele in Rous sarcoma regression (Cutting et al. 1981) has been discussed above. Specific interaction between MHC haplotypes in response to Rous sarcoma was also observed by Brown and co-workers (1982) within noninbred line UNH 105 segregating for B^{23}, B^{24}, and B^{26}. Tests of the six genotypes showed that one genotype, B^{23}/B^{26}, had significantly greater regression ability than any of the other five genotypes. It is unlikely, however, that interaction between non-MHC genes contributed to the superior regressor ability of B^{23}/B^{26} chickens. The population studied was noninbred and was produced from a total of 28 sires and 112 dams. Any segregating non-MHC genes contributing to regression would therefore be expected to be distributed approximately equally among all six *B* complex genotypes.

The present research, coupled with that of Gilmour and co-workers (1983), shows that in homozygous B^2/B^2 chickens, lymphocyte surface alloantigen loci *Ly-4*, *Th-1*, and *Bu-1* participate in the response to Rous sarcomas. Thus, evidence is accumulating to suggest that both MHC and non-MHC genes contribute to the regression of these tumors.

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