Research Note: Rous sarcoma growth differs among congenic lines containing major histocompatibility (*B*) complex recombinants

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ABSTRACT New arrangements of chicken major histocompatibility complex (**MHC**) class I BF and class IV BG genes are created through recombination. Characterizing the immune responses of such recombinants reveals genes or gene regions that contribute to immunity. Inbred Line UCD 003 (B17B17) served as the genetic background for congenic lines, each containing a unique MHC recombinant. After an initial cross to introduce a specific recombinant, 10 backcrosses to the inbred line produced lines with 99.9% genetic uniformity. The current study compared Rous sarcoma virus (**RSV**) tumor growth in 5 congenic lines homozygous for MHC recombinants (003.R1 = BF24-BG23, 003.R2 = BF2-BG23, 003.R4 = BF2-BG23, 003.R5 = BF21-BG19, and 003.R13 = BF17-BG23). Two experiments used a total of 70 birds from the 5 congenic lines inoculated with 20 pock forming units of RSV subgroup C at 6 wk of age. Tumor size was scored 6 times over 10 wk postinoculation followed by assignment of a tumor profile index (**TPI**) based on the tumor size scores. Tumor growth over time and rank transformed TPI values were analyzed by least squares ANOVA. Tumor size increased over the experimental period in all genotypes through 4 wk postinoculation. After this time, tumor size increased in Lines 003.R1, plateaued in Lines 003.R2, 003.R4, and 003.R13, and declined in 003.R5. Tumor growth over time was significantly lower in Line 003.R5 compared with all other genotypes. In addition, Line 003.R5 chickens had significantly lower TPI values compared with Lines 003.R2, 003.R4, and 003.R13. The TPI of Line 003.R1 did not differ significantly from any of the other genotypes. The BF21 in Line 003.R5 produced a greater response against subgroup C RSV tumors than did BF24, found in 003.R1; BF2 found in 003.R2 and R4 as well as BF17 found in 003.R13.

Key words: immunity, recombination, Rous sarcoma virus, tumor

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INTRODUCTION

The ability to mount an immune response against antigenic challenge is critical for individual survival. Mammals respond to a range of antigens, even those substances encountered for the first time. The multigene major histocompatibility complex (**MHC**) regulates these responses. In contrast, the chicken MHC has a single class I (*B*F) gene with high expression. This one MHC class I (*B*F) gene is the basis for the association between MHC type and disease responses. Early experiments related Marek's disease and Rous sarcoma outcome to MHC type. Further studies expanded the range of MHC control of responses against pathogens and nonpathogenic antigens (Miller and Taylor, 2016; Kaufman, 2018).

The oncogenic retrovirus Rous sarcoma virus (**RSV**) has 3 subgroups (A, B, C) that produce tumors in susceptible chickens. Each subgroup has cell surface receptors encoded by a single gene with 2 alleles: receptor positive, dominant and receptor negative, recessive. The presence of the specific receptor is required for the virus to enter a cell (Taylor, 2004). Tumor growth is affected by host genes including the MHC, a related polymorphic system, MHC-Y, and non-MHC alloantigens (LePage et al., 2000; Taylor, 2004).

The MHC influence on RSV-induced tumors is well documented. Tumors regress, progress, or remain static depending on the MHC type of the host. In addition, growth of *v-src* DNA tumors, produced by oncogene

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DNA injection, is controlled by the MHC. Outbred, inbred and congenic stocks have been tested to identify the MHC homozygous types as regressors or progressors. For example, tumor growth is lower in chickens having haplotypes *B*2, *B*6, *B*21 and *B*23, intermediate in *B*19, and higher in B5, B13, *B*17, and *B*24 (Taylor, 2004). Some MHC homozygotes types as well as some MHC heterozygous combinations have an intermediate response in which tumors remain present without a change in size. A second exposure to the original inoculum, either virus or *v-src* DNA shows protection against a second tumor in MHC regressor genotypes (Taylor, 2004).

Although multiple instances of recombination within the chicken MHC have been described, no class I (BF) and class II (BL) gene recombinants have been detected because of the tight linkage between the BF and BL. Examination of DNA sequences indicated that such recombination may have occurred in the more distant past. The BG genes are separated from BF/BL by sufficient distance to permit recombination. Chickens bearing MHC recombinants assisted with the analysis of immune responses. Studies using these recombinants allowed control of responses against natural or artificial antigens to be assigned to a specific genome region (Miller and Taylor, 2016; Kaufman, 2018).

Congenic lines are valuable resources to confirm that specific genes control certain outcomes. Inbred Line UCD 003 was used as the genetic background for congenic lines with individual MHC recombinants. Each recombination was unique, arising from an independent event, even though some recombinants had serological similarity. Sequence analysis confirmed the singular nature of each recombinant. Six lines were produced by multiple backcrosses of each recombinant to the inbred line. These lines, homozygous for a single recombinant, have response differences against Marek's disease, RSV, *Eimeria tenella*, sheep red blood cells, and bovine red blood cells (Clare et al., 1989; Schulten et al., 2007, 2009; Goto et al., 2009; Wilkinson et al., 2020).

This study assessed subgroup C RSV tumor growth and tumor outcome in 5 congenic lines homozygous for MHC recombinants. Previous experiments with these congenic lines examined RSV subgroup A tumor growth. In addition, a new congenic line, 003.R13 (BF17-BG23) was included after recombinant R13 arose in the R1 (BF24-BG23) tenth backcross generation to the inbred Line UCD 003 background. The congenic lines have calculated 99.9% background gene similarity which excluded non-MHC gene effects.

MATERIALS AND METHODS

Stock

Five congenic lines, each homozygous for a different recombinant of the major histocompatibility (*B*) complex on the inbred Line UCD 003 (*B*17*B*17) background, were used. The 5 lines, 003.R1, 003.R2, 003.R4, 003.R5 and 003.R13 were abbreviated to their specific homozygous genotype R1R1, R2R2, R4R4, R5R5 and R13R13. The specific genotypes for each recombinant were confirmed by serology (Schulten et al., 2007, 2009). Four lines (R1R1, R2R2, R4R4, R5R5) were produced by ten backcrosses to the inbred Line UCD 003 as described (Schulten et al., 2007). The R13R13 line, contained a new recombinant that arose in one male from the tenth backcross generation (Wilkinson et al., 2020). Another backcross was required to increase the number of R13 birds available for production of R13R13 homozygous birds. Therefore, the genome of all 5 lines was calculated to be at least 99.9% identical.

Five to 8 dams and a single sire were housed in pens to produce fertile eggs for each line. Eggs were collected for 2 wk followed by standard incubation procedures. When the chicks hatched, each received an individual wing band and Marek's disease vaccine. Another vaccine against Newcastle-bronchitis was given when the chicks were 10-day-old. Heated brooders were used for housing the chicks during the first 6 wk of the experiments. After 6 wk birds were transferred to grower cages for group housing. Food and water that were free of antibiotics were available continuously over the experiments. The appropriate Institutional Animal Care and Use Committee approved all described procedures.

Genotype Identification

Hemagglutination assays with separate polyclonal antisera specific for the B-F and B-G region of each recombinant were used to confirm the genotype for each bird. Assays followed established procedures (Schulten et al., 2007; Wilkinson et al., 2020).

Virus Inoculation and Tumor Measurement

A total 70 chicks of the 5 congenic lines differing only by their recombinant MHC-B complexes were produced in 2 hatches. In total, each line was represented by 11 to 15 individuals. At 6 wk of age, the chicks received wing-web injections of 20 pock forming units RSV (subgroup C). Tumor size was scored at 2, 3, 4, 6, 8, and 10 wk postinoculation using the described scale (Schulten et al., 2009). Scores for tumors range on a scale using the values 0 = no palpable tumor; 1= small tumor up to 0.5 cm diameter; 2 = tumor > 0.5 up to 1.2 cm diameter; 3 = tumor>1.2 up to 1/2 wing-web area; 4 = tumor> 1/2 wing-web area, but < entire wing web; 5 = tumor filling the entire wing-web; 6 = massive tumor extended beyond wing-web; and 7 = mortality during the 70 d experiment. A tumor profile index (**TPI**) was assigned to each chicken from the six tumor size scores where 1 = complete regression by 70 d or a decreasing slope or complete regression by 56 d followed by recurrence; 2 = general upward trend, a plateau or slight regression after 56 d; 3 = terminal tumor after 42 d postinoculation; 4 = terminal tumor between 29 and 42 d post inoculation; and 5 = terminal tumor by 28 d postinoculation (Schulten et al., 2009).

Statistical Analysis

In each analysis, trial and *B* genotype were the main effects in the statistical model. Tumor size scores over time were evaluated using a repeated measures ANOVA. Rank transformation was applied to all TPI values, which were followed by ANOVA as described (Schulten et al., 2009). Fisher's protected LSD was used to determine differences between recombinant groups.

RESULTS AND DISCUSSION

Six-wk-old congenic lines, each having a different MHC recombinant, were injected with 20 pock forming units of RSV subgroup C. The Line UCD 003 background of the congenic lines is susceptible to cellular infection with the specific viral subgroup. Eighty-one percent of all birds injected developed tumors. Tumor production was consistent across all five lines such that no line had an excess of birds that failed to initiate a tumor. A chi-square test for homogeneity found no line difference in the distribution of birds with or without tumors (data not shown).

Tumor size increased during the first three evaluations at 2, 3, and 4 wk postinoculation. After 4 wk postinoculation, the tumor size declined in Line 003.R5, plateaued in Lines 003.R2, and 003.R4 and increased in Lines 003.R1 and 003.R13. Line 003.R5 birds had a tumor growth plateau (Figure 1) at a lower tumor size compared with the tumor size in the other 4 lines. The tumor growth curve for Line UCD 003.R5 was lower than the other 4 lines and that difference was significant (Figure 1). None of the lines had complete tumor regression by the end of the experiment at 70 d postinoculation.

The TPI values are shown in Figure 2. Lines 003.R2 (4.0 ± 0.5) and 003.R4 (4.0 ± 0.4) had the highest TPI. The next line in decreasing TPI was 003.R13 (3.4 ± 0.3) followed by Line 003.R1 TPI (3.0 ± 0.5). Line 003.R5 had the lowest TPI (2.0 ± 0.1) that differed significantly from Lines 003.R2, 003.R4 and 003.R13. No significant

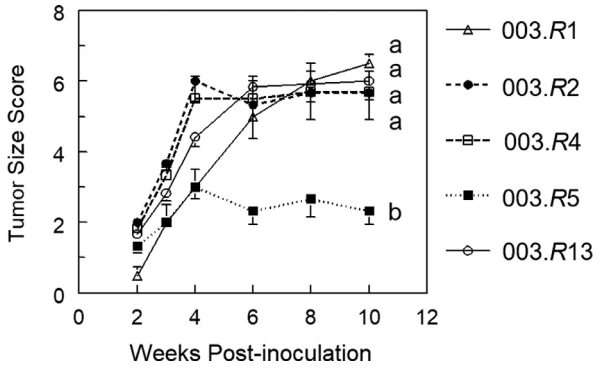

Figure 1. Mean (± SEM) tumor size scores in 5 UCD 003 congenic lines containing MHC recombinants after 20 pfu injection of Rous sarcoma virus (RSV) subgroup C at 6 wk of age. Tumor size was scored 6 times over a 10-wk period postinoculation. Overall tumor growth in R5R5 differed significantly ($P < 0.05$) from all other lines. Tumor growth curves without a common letter differ significantly ($P > 0.05$) over time.

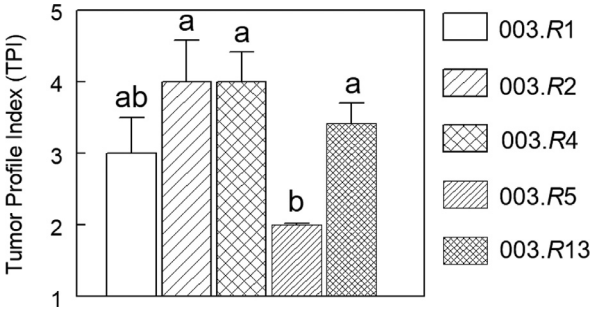

Figure 2. Mean (± SEM) tumor profile indices (TPI) in 5 UCD 003 congenic lines containing MHC recombinants after 20 pfu injection of Rous sarcoma virus (RSV) subgroup C at 6 weeks of age. Each bird was assigned a TPI based on the tumor size scores' pattern over a 10-wk period postinoculation. TPI bars without a common letter differ significantly ($P < 0.05$).

difference was detected in the comparison of 003.R1 with the other 4 congenic lines.

Previous experiments compared tumor growth in congenic lines carrying major histocompatibility complex recombinants. Tumors were induced by injection of RSV subgroup A. Four of the congenic lines (003.R1, 003.R2, 003.R4, 003.R5) were used in that study as well as the current test (Schulten et al., 2009). Subgroup A RSV tumor growth differed between Lines 003.R2 and 003.R4. The 2 recombinants differ by the presence of a 255 bp insert in the 003.R4 BG1 3' UTR that is absent in of 003.R2. The BG1 insert influenced RSV subgroup A, but not subgroup C tumor growth.

Tumor growth and tumor outcome may differ according to virus subgroup. Lines GB-1 (*B*13*B*13) regressed tumors produced by one subgroup B RSV and 2 subgroup C viruses. On the other hand, Line GB-2 (*B*6*B*6) progressed these tumors. Tumors caused by another subgroup B virus regressed in both lines. An inbred line cross ($15I_5 \times 6_3$) F_2 segregated for *B*2*B*2, *B*2*B*15, and *B*15*B*15 genotypes. The first 2 genotypes regressed tumors produced by subgroup A, B, and C viruses. Genotype *B*15*B*15 progressed tumors but subgroup C virus tumors had lower growth (Taylor, 2004).

Line 003.R1 progressed tumors either from RSV subgroup A (Schulten et al., 2009) or subgroup C (current study). This result was expected because the *B*F24 found in the recombinant was associated with tumor progression according to multiple reports (Taylor, 2004). Line 003.R5 (*B*F21-*B*G19) had the lowest TPI after injection of RSV subgroup A or subgroup C. Haplotype *B*21 was associated with tumor regression (Taylor, 2004; Schulten et al., 2009).

The B17 haplotype has BF17 and BG17 compared with the new R13 recombinant which has BF17 and BG23. Haplotype B23 controls more regressive tumor growth (Taylor, 2004). However, tumor growth was progressive in R13. A previous study showed that B17B17 birds had increasing tumor growth following injection of 15 or 30 pock-forming units of RSV subgroup A (Senseney et al., 2000). The presence of BG23 did not impact tumor growth based in previous studies. Schulten et al. (2009) found no tumor growth difference

between 003.R5 (BF21-BG19) and 003.R6 (BF21-BG23) after inoculation with RSV subgroup A. These results suggested that BG genes did affect RSV-induced tumor size and outcome.

Each MHC recombinant contained in the congenic lines arose from an independent event, meaning that the chromosomal breakpoint and the genes contained therein are different. One example occurred in recombinants R2 and R4 which have a genetic alteration as well as differential responses against RSV and Marek's disease (Goto et al., 2009; Schulten et al., 2009). Similar tumor growth in the current study's 4 recombinants containing BG23 (003.R1, 003.R2, 003.R4, 003.R13) showed that none of the B23 recombination points included genes that have a positive impact on RSV C tumors.

In summary, lower growth and TPI of RSV subgroup C tumors in Line 003.R5 were significant compared with 4 other congenic lines with MHC recombinants 003.R1, 003.R2, 003.R4, and 003.R13. Line 003.R13 chickens congenic for R13 (BF17-BG23), a new MHC recombinant, showed progressive tumor growth. Tumor growth and TPI in 3 other congenic lines 003.R1, 003.R2, and 003.R4 did not differ from 003.R13 or from each other. The congenic lines containing MHC recombinants are useful to dissect control of immune responses.

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DISCLOSURES

The authors declare no conflict of interest.

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