Rfp-Y region polymorphism and Marek's disease resistance in multitrait immunocompetence-selected chicken lines

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Abstract
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Keywords
chicken, selection, multitrait immunocompetence, Rfp-Y, Marek's disease

Disciplines
Agriculture | Animal Sciences | Genetics | Poultry or Avian Science

Comments

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**ABSTRACT** Although the influence of the chicken classical MHC in resistance to many diseases is well established, the role of the recently identified, genetically independent, MHC-like region known as Rfp-Y is unclear. The objectives of this study were to analyze the frequencies of DNA polymorphisms of the Rfp-Y region in White Leghorn lines, which were divergently selected in replicate for multitrait immunocompetence, and to determine the association of these polymorphisms with Marek’s disease (MD) resistance. Chicks, either with or without herpes virus of turkey (HVT) vaccination, were challenged with 500 ffu of a very virulent Marek’s disease virus (Md5) at 2 d of age. The MD-related data were collected for 10 wk. PvuII-digested genomic DNA was hybridized with an Rfp-Y region-specific probe, 18.1. Three Rfp-Y polymorphisms were observed. The frequency of one Rfp-Y polymorphism was significantly different between divergently selected multitrait immunocompetence lines in one replicate only; therefore, the impact of multitrait immunocompetence selection on Rfp-Y polymorphisms is inconclusive. The PvuII defined Rfp-Y region polymorphisms had no association with either innate or vaccine-induced MD resistance to Md5 virus challenge.

(Key words: chicken, selection, multitrait immunocompetence, Rfp-Y, Marek’s disease)

1998 Poultry Science 77:538–541

**INTRODUCTION**

The classical MHC in the chicken, known as the B complex, was first described as a blood group system by Briles et al. (1950) and was later identified as the chicken MHC by Schierman and Nordskog (1961). The chicken classical MHC consists of class I, class II, and class IV genes (B-F, B-L, and B-G, respectively), which code for highly polymorphic cell surface antigens (Kaufman and Lamont, 1996). A second MHC-like chromosomal region was described in a study by Briles et al. (1993). This newly identified region, named Rfp-Y, contains polymorphic class I and class II genes, a c-type lectin gene, and other genes (Miller et al., 1994, 1996). Although initial studies failed to show genetic linkage between 400 DNA markers and Rfp-Y, physical mapping by Miller et al. (1996) demonstrated that the Rfp-Y chromosomal segment is located on the same microchromosome as classical MHC (chromosome 16).

Associations between the classical MHC and Marek’s disease (MD) resistance in inbred and commercial populations have been well documented (Hansen et al., 1967; Briles et al., 1977; Gavora et al., 1986). The role the Rfp-Y region in disease resistance is not as well established. Recently, Wakenell et al. (1996) reported that a Rfp-Y haplotype was significantly associated with the outcome of MD virus infection; however, Bacon et al. (1996) reported that Rfp-Y haplotypes identified among N and P lines had no significant effect on MD resistance.

The major objectives of this study were to determine the effect of divergent selection for multitrait immunocompetence on Rfp-Y region polymorphisms and the association of Rfp-Y polymorphisms with MD resistance.

**MATERIALS AND METHODS**

**Chicken Lines and MD Challenge**

The chicken lines were derived from a White Leghorn strain (Ottawa Strain 7) and had been divergently selected (High and Low) for nine generations in replicates (1 and 2) for multitrait immunocompetence (Cheng et al., 1991; Kean et al., 1994), resulting in four lines (1High, 1Low, 2High, and 2Low). Sixty chicks per line (a total of 240 chicks) were inoculated i.p. with 500 ffu of Md5 virus.
(Witter, 1983) at 2 d of age. Half of the chicks in each line had received herpes virus of turkeys (HVT) vaccine at 1 d of age. Chicks were provided ad libitum access to feed and water. Morbidity symptoms and mortality were recorded daily. Chicks with severe symptoms of MD were euthanatized. During the experimental period, dead and euthanatized chicks were necropsied daily and visually examined for MD-associated lesions and tumors. At 10 wk of age, the remaining chicks were euthanatized and visually examined for MD-associated lesions.

**DNA Analysis**

Genomic DNA was isolated from whole blood cells of each chick (Burke and Bruford, 1987), digested with *Pvu*II restriction enzyme, size separated on 0.8% agarose gel, and transferred to a nylon membrane. The $^{32}$P-labeled 18.1 probe (a 1.6-kb genomic fragment adjacent to the 17.5 lectin gene in the *Rfp-Y* chromosomal segment, Miller et al., 1994) was used to investigate restriction polymorphism patterns of the *Rfp-Y* region. Previous studies with the 18.1 probe and three restriction enzymes ($Pst$I, $Bgl$I, and *Pvu*II) indicated that the restriction fragment polymorphisms revealed by the 18.1 probe cosegregated with the restriction fragment polymorphisms defining *Rfp-Y* (Miller et al., 1994), and thus the 18.1 probe was used to investigate *Rfp-Y* region polymorphism among chicken lines used in this study. For autoradiography, membranes were exposed to x-ray film following washes at 65°C with 0.5×SSC (20 min, twice) and 0.1×SSC (20 min, twice).

**Data Analysis**

Based upon clinical and necropsy data, birds were categorized as either positive for MD (birds with tumor or MD-related symptoms or both) or negative for MD (with no visual evidence of MD in live or necropsied birds). Data were analyzed by chi-square tests with appropriate contingency tables, testing the null hypotheses of: no difference in frequency of *Rfp-Y* types due to selection or replication, or no association of *Rfp-Y* types with MD incidence. Because there was a significant ($P < 0.01$) difference in MD (7.0 vs 43.9%) and tumor (10.4 vs 50.0%) incidence between vaccinated and unvaccinated groups, respectively, the associations of *Rfp-Y* type with MD and tumor incidence were separately analyzed by vaccination status.

**RESULTS AND DISCUSSION**

There were three high-intensity, consistently scorable bands observed, of which two (Band 1 and Band 2) were polymorphic (Figure 1). Based on the polymorphic band patterns, birds were classified into one of three *Rfp-Y* types (A, B, C). It is probable that birds with A or B *Rfp-Y* type are homozygous and *Rfp-Y* type C represents the heterozygote. Because birds from a single generation were used in this study, however, additional study with multiple generations would be needed to confirm this hypothesis. The *Rfp-Y* bands and patterns observed with the 18.1 probe do not cosegregate with any of the classical MHC class II bands (data not shown) observed with a genomic classical MHC class II probe (CCII-7-1, Xu et al., 1989).

The frequencies of *Rfp-Y* types by selection direction, line, and replicate in the four multitrait immunocompetence lines are listed in Table 1. In Replicate 1, but not in Replicate 2, the frequency of *Rfp-Y* type A was significantly higher in the High line ($P < 0.003$) than in the Low line. The frequency of *Rfp-Y* types B and C were not significantly different between High and Low lines in either replicate. Between replicates, the frequencies of *Rfp-Y* types A and B were significantly different. A combined analysis of High lines and Low lines from Replicates 1 and 2 suggested that the frequency of all three *Rfp-Y* types did not differ by selection direction.
TABLE 1. Frequency of Rfp-Y types among multitrait immunocompetence-selected lines by line, selection direction, and replicate

<table>
<thead>
<tr>
<th>Variable</th>
<th>n(^1)</th>
<th>A (%)</th>
<th>B (%)</th>
<th>C (%)</th>
<th>A value</th>
<th>B value</th>
<th>C value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1High</td>
<td>60</td>
<td>50.0(^a)</td>
<td>10.0(^b)</td>
<td>40.0(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1Low</td>
<td>59</td>
<td>23.7(^a)</td>
<td>22.0(^a)</td>
<td>54.2(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(P) value</td>
<td></td>
<td>0.00</td>
<td>0.07</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2High</td>
<td>55</td>
<td>47.3(^a)</td>
<td>3.6(^b)</td>
<td>49.1(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2Low</td>
<td>55</td>
<td>56.4(^a)</td>
<td>1.8(^b)</td>
<td>41.8(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(P) value</td>
<td></td>
<td>0.34</td>
<td>0.56</td>
<td>0.44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selection direction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>115</td>
<td>48.9</td>
<td>7.0</td>
<td>44.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>114</td>
<td>39.5</td>
<td>12.3</td>
<td>48.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(P) value</td>
<td></td>
<td>0.16</td>
<td>0.17</td>
<td>0.55</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Replicate 1</td>
<td>119</td>
<td>37.0</td>
<td>16.0</td>
<td>47.0</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Replicate 2</td>
<td>110</td>
<td>51.8</td>
<td>2.7</td>
<td>45.5</td>
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<td></td>
<td></td>
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<tr>
<td>(P) value</td>
<td></td>
<td>0.02</td>
<td>0.00</td>
<td>0.81</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a,b}\)Values within each column with no common superscript differ significantly (\(P < 0.05\)).

\(^{1}\)Total number tested.

Dunnington et al. (1996) illustrates and emphasizes the importance of replication of populations in genetic selection for accurate interpretation of data. If the lines used in this study had not been replicated, one might conclude either that selection for multitrait immunocompetence altered the frequency of Rfp-Y type (results from Replicate 1) or that selection had no influence on Rfp-Y type frequency (results from Replicate 2). Because of the inconsistencies between replicates, no definitive conclusion can be made about immunocompetence selection effect on Rfp-Y type and, thus, the data available from this study with replicated populations helps to guard against acceptance of false-positive results.

Previously, selection for multitrait immune response was shown to have no effect on incidence of MD development after a challenge with the Md5 virus strain (Lamont et al., 1996). Therefore, the lines were pooled and association of Rfp-Y type with MD development was analyzed based on vaccination status of all experimental animals (Table 2). As expected, the incidence of MD was significantly lower in the vaccinated group than in the unvaccinated group for all three Rfp-Y types. There were, however, no significant differences among the three Rfp-Y types and MD incidence. The results suggest that the three Rfp-Y types defined in these lines by PvuII digestion were not associated with MD development. Additional restriction enzyme digestions might define additional polymorphisms with different relationship to MD.

Two studies of associations between Rfp-Y haplotypes and MD resistance have been reported (Wakenell et al., 1996; Bacon et al., 1996). Wakenell et al. (1996) reported that Rfp-Y is significantly associated with the outcome of MD virus infection, based on a study with Rfp-Y typed chicks from pedigreed and unpedigreed populations that were challenged with the RB1B strain of MD virus. Bacon and collaborators (1996), however, reported no significant associations between Rfp-Y haplotype and MD resistance in MD resistant and susceptible lines in a study with Rfp-Y typed inbred chicks that were challenged with the JM strain of MD virus. The results from our study suggest that Rfp-Y region polymorphisms may not influence resistance to Md5 strain of MD virus in either vaccinated or unvaccinated birds derived from Ottawa Strain 7. Because virus virulence and host genotype play a major role in the outcome of MD viral infection, the lack of agreement on the role of Rfp-Y type on MD resistance among these studies may be because of the virus strain used for challenge or the genotype (e.g., technique used for assigning Rfp-Y type, specific Rfp-Y haplotypes, specific MHC alleles, or genetic background) of the experimental birds.

TABLE 2. Rfp-Y type and incidence of Marek's disease (MD) among multitrait immunocompetence lines by vaccination status

<table>
<thead>
<tr>
<th>Variable</th>
<th>A (%)</th>
<th>B (%)</th>
<th>C (%)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated</td>
<td>15.1%</td>
<td>8.3%</td>
<td>6.0%</td>
<td>0.31</td>
</tr>
<tr>
<td>Unvaccinated</td>
<td>47.9%</td>
<td>50.0%</td>
<td>51.8%</td>
<td>0.93</td>
</tr>
</tbody>
</table>

\(^{1}\)Percentage (affected/total number) of birds classified as each Rfp-Y type.

ACKNOWLEDGMENTS

The authors thank C. Auffray and R. Zoorob, CNRS-IRC/IFC, Villejuif, France, for providing the 18.1 probe, and Michael Kaiser, Iowa State University, for assistance with the MD trials.

REFERENCES


