ASSOCIATIONS BETWEEN THE B AND R BLOOD GROUP LOCi AND RESISTANCE TO CERTAIN ONCOGENIC VIRUSES IN CHICKENS

Associations entre les loci des groupes sanguins B et R et la résistance à certains virus oncogéniques chez les poules

Beziehungen zwischen den B und R Blutgruppenloci und Widerstand gegen bestimmten onkogenischen Viren in Hühnern

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The B blood group system in chickens was recognized as being highly polymorphic at the time of its initial discovery (Briles, McGibbon and Irwin, 1948, 1950). Over the next decade the B system was studied in numerous populations both inbred and noninbred. Each population, almost without exception, possessed two or more B alleles. Performance data from several populations revealed that the B blood group genotypes were rather frequently associated with certain physiological traits — primarily hatchability, livability and egg production (for reviews of work during this period see Gilmour, 1960; Briles, 1960 and 1964). Essentially, it had become clear that B alleles often differed in their overall effects on fitness; that livability effects may differ considerable in the juvenile and adult periods; and that the magnitude of the differences in livability between B genotypes were most pronounced when the total mortality was high. These observations, together with the fact that a high proportion of mortality in chickens was accounted for by leukosis, suggested that the B locus might be specifically related in some way to genetic resistance to this disease. During the mid-1960s research advances into the etiology and pathology of leukosis (avian leukosis complex) revealed that it consisted of two distinct diseases, Marek's disease and lymphoid leukosis, each caused by a different group of viruses and each having certain distinguishing pathological features (for reviews see Biggs, 1973; Purchase, 1973). With this advance in knowledge it became feasible to challenge chickens possessing different B alleles with appropriate inocula to test for specific effects on susceptibility.

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The first study of this nature was reported in 1967 by Hansen, Van Zandt and Law. Over a two-year period 854 hybrid chicks possessing $B^o$ or $B^2$ were challenged with 0.1 ml suspension of macerated tissue from chickens showing gross lesions of Marek's disease. In order to avoid confounding blood group genotype and family structure in the transmission of the two blood group alleles to progeny under test, the chicks were sired by heterozygous males, $B^o B^2$, and by sets of brothers homozygous for opposite alleles, $B^o B^o$ and $B^2 B^2$. The mortality of the $B^o$ and $B^2$ chicks to 10 (or 12) weeks of age in a total of five challenge experiments was 29.0% and 16.0%, respectively. Laboratory postmortem examination confirmed that the mortality was due to Marek's disease. In a natural exposure study from the same matings a total of 3982 chicks were placed at 24 separate farm locations. Mortality of $B^o$ and $B^2$ to 22 weeks of age on 10 farms having a considerable incidence of Marek's disease was 12.4% and 6.5%, respectively; at the other 14 farms, where the incidence of Marek's disease was considered to be minimal, the mortality to 22 weeks of the corresponding types was 4.5% and 2.6%, respectively. These data indicate that the mortality of those chicks inheriting $B^o$ is roughly twice that of those receiving $B^2$, irrespective of artificial or natural exposure.

Association between $B$ alleles and leukosis susceptibility in two related Leghorn lines was detected by Brewer, Moore and Johnson (1968). In a natural exposure experiment they found that the percent of total leukosis mortality associated with each of five $B$ alleles ranged from zero to 14%. In a total of three experiments in which chicks were artificially exposed to Marek's disease (GA isolate), the mortality to 10 weeks of age for the same five $B$ alleles ranked similarly within a range of 3.8% to 8.2%.

Briales and Oleson (1971) reported a highly significant deficiency of one $B$ allele segregating in fully blood typed sisters suffering from a severe natural outbreak of Marek's disease. Pedigreed females produced by crossing two inbred lines were typed for 11 blood group systems in their sixth month of lay. The families supplying pertinent data were from $B'B'$ or $B'B'$ sires of DeKalb line 2 and $B'B'$ dams of DeKalb line 3. It was noticed in assigning genotypes from the typing information that the $B'B'$ daughters from both types of sires occurred in a frequency distinctly lower than the 0.50 expected on the basis of chance segregation of $B'B'$ or $B'B'$. A summary of the total data shows that the $B'$ allele was present in only 29.0% of 162 progeny typed from $B'B'$ sires and in only 28.8% of 111 progeny from $B'B'$ sires; the remaining offspring (71.1% and 71.2%, respectively) possessed either $B'$ or $B'$ depending on the genotype of the respective sires. This group of birds was hatched in 1969 and suffered considerable Marek's mortality, resulting in 58% depletion by the time the birds were blood typed at one year of age.

Six previous generations of this cross had been produced during the years 1961 through 1967 and had been typed at an age comparable to that of the above group. During these earlier years mortality from all causes ranged from about five to seven percent, and Marek's disease had not been specifically recognized as being present on the farm during this period. Over these generations the $B'B'$ and $B'B'$ progeny from $B'B'$ sires numbered 290 and 320, respectively; similarly, $B'B'$ and $B'B'$ progeny from $B'B'$ sires numbered 371 and 374, respectively. Chi-square tests for homogeneity failed to disclose any genotype x year interactions over
this 1961-1967 period. Pooling these genotype distributions for the earlier period and comparing them with the distributions present in the same single-cross blood typed in 1970 showed very highly significant genotype × year interactions among the daughters of both the B'B (Table 1) and B'B (Table 2) sires. These data indicate that under the stress of Marek's disease, as experienced in the latter period, the relative survival of progeny inheriting either B or B' was approximately 2.5 times that of their sisters inheriting B'.

### TABLE 1

**Number of B'B and B'B progeny recovered in 1970 compared to 1961-1966 from B'B line 2 sires × B'B line 3 dams**

<table>
<thead>
<tr>
<th>Year</th>
<th>B'B</th>
<th>B'B</th>
<th>d.f.</th>
<th>Chi-square</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970</td>
<td>47</td>
<td>115</td>
<td>1</td>
<td>28.5432</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>1961-1966</td>
<td>290</td>
<td>320</td>
<td>1</td>
<td>1.4754</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>337</td>
<td>435</td>
<td>2</td>
<td>30.0186</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pooled</td>
<td>337</td>
<td>435</td>
<td>1</td>
<td>12.4404</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Interaction</td>
<td></td>
<td></td>
<td>1</td>
<td>17.5782</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

### TABLE 2

**Number of B'B and B'B progeny recovered in 1970 compared to 1961-1966 from B'B line 2 sires × B'B line 3 dams**

<table>
<thead>
<tr>
<th>Year</th>
<th>B'B</th>
<th>B'B</th>
<th>d.f.</th>
<th>Chi-square</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970</td>
<td>32</td>
<td>79</td>
<td>1</td>
<td>19.9009</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>1961-1966</td>
<td>371</td>
<td>374</td>
<td>1</td>
<td>0.0121</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>403</td>
<td>453</td>
<td>2</td>
<td>19.9130</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pooled</td>
<td></td>
<td></td>
<td>1</td>
<td>2.9206</td>
<td></td>
</tr>
<tr>
<td>Interaction</td>
<td></td>
<td></td>
<td>1</td>
<td>16.9924</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

It is reasonable to expect survival values of the magnitude observed above to result in gene frequency changes as a result of natural selection in parental line 2, for it was subjected also to the natural outbreak of Marek's disease. Fortunately, from the standpoint of contributing meaningful data, this line had been separated into three genetic isolates just prior to the severe outbreak at the time of Marek's disease; each isolate was under independent artificial selection including a challenge test of progeny for resistance to Marek's disease, during the 1969-1971 period when natural exposure was also high. The changes in allele frequencies for B', B' and B' in adult populations typed over this period are indicated in Figure 1. Sub-line a was only typed in 1969 and 1971; whereas sublines b and c were typed in 1969, 1970 and 1971. In conformity with the differences in survival values for the three alleles observed above for the single-cross progeny the low survival allele, B', decreased in frequency in each of the three sub-lines while both of the high-survival alleles, B' and B', consistently increased in frequency in each of the three sub-lines.
Starting with a highly variable genetic stock having a demonstrated Marek's mortality of 51.1%, Cole (1968) selected on the basis of resistance to mortality following challenge with the agent of Marek's disease. In two generations of selection he was able to produce resistant and susceptible lines with mortalities of 12.9% and 90.7%, respectively. Recently the current generations of these two lines were typed for 12 blood group systems (Briles and Stone, 1974). Of the 12 loci involved only the B shows distinct frequency changes in the two lines. The B locus in the resistant line is almost completely homozygous for one particular allele (0.99 frequency) which is probably missing from the line selected for susceptibility. The latter line has a minimum of three B alleles, only one of which was present the resistant line (in 2 of 109 birds tested). The current generation of the stock from which Cole made his original selections has also been typed and found to possess essentially a composite of the alleles present in the resistant
and susceptible lines developed by Cole. Thus, it appears that in selecting for resistance to Marek's disease selection pressure was inadvertently applied to a particular B allele. A study designed to determine the relative effect on survival following challenge with Marek's disease for the B allele in the resistant line versus the several B alleles in the susceptible line is currently underway.

The means by which certain B alleles may convey resistance to Marek's disease is unknown. The histocompatibility loci in several mammalian species (e.g., mouse and guinea pig) have been found to be associated with immune response to specific antigens (Benacerraf and McDevitt, 1972). The B locus in chickens was first established as having strong histocompatibility effects by Schierman and Nordskog (1961). Subsequent work (Jaffe and McDermid, 1962; Gilmour, 1963; Gleason and Fanguy, 1964) has firmly established the B locus as the major histocompatibility system in the chicken. These facts suggest that resistance to Marek's disease associated with the B alleles may be conferred through the immune response of the host—possibly against the tumor cells themselves. Knowledge of the nature of the effect of the B system on resistance to the onogenic herpes-virus of Marek's disease is of importance both from the standpoints of a more complete understanding of the nature of genetic resistance to tumors and allowing the practical breeder to increase Marek's resistance in a manner compatible with simultaneous selection for other economic traits.

An antigen of a more recently detected blood group system, R, appears to act as a cell-surface receptor for the B subgroup of avian leukosis-sarcoma viruses (Crittenden, Briles and Stone, 1970; Crittenden and Briles, 1971). This was discovered by testing the erythrocytes of individual adult chickens of known A and B susceptibility genotypes with a large panel of blood typing reagents. Examination of the distribution of blood group classes among the susceptibility types (Table 3) revealed that all 35 individuals possessing the gene b' for susceptibility to subgroup B virus (19 b' b' and 16 b' b) possessed R, (blood group class R')—while the five birds homozygous for the resistant allele b did not have the R antigen (blood group class rr).

This relationship was confirmed by subjecting embryos from the mating b' b' x b' b' to simultaneous CAM tests for susceptibility and hemagglutination tests.
for the R₁ antigen. There were 191 embryos that were susceptible and R₁-positive and 194 embryos resistant and R₁-negative. Two additional embryos were classified as R₁-positive and resistant; these two exceptional embryos could have resulted from recombination but more probably represent technical error as subsequent work has failed to disclose additional discrepancies. In some lines of chickens two alleles for B subgroup susceptibility have been found—one associated with the occurrence of the R₁ antigen (b¹) and the other with the absence of it (b²) (Crittenden, et al 1970, 1973). In fact, the line in which the R₁ reagent was developed possessed all three alleles—b₁⁺, b₂⁻ and b⁻. Further studies are being carried out to develop isohemagglutinating reagents for other antigens of the R system and to discover isoantigens that may be acting as receptors for the A and C subgroups of avian leukosis-sarcoma viruses.

**SUMMARY**

Resistance to Marek's disease has been shown to be associated with the inheritance of particular alleles of the highly polymorphic B blood group locus in chickens. Under conditions of both natural and artificial exposure one group of investigators found that the mortality of chickens possessing one allele may be twice that of those possessing a contrasting allele. For example, the brooding period mortality of chicks inheriting B¹⁺ or B²⁻ in challenge experiments was 29% and 16%, respectively. Another group of investigators observed a differential depletion of B blood group genotypes among progeny from heterozygous sires following a severe natural outbreak of Marek's disease—progeny inheriting contrasting alleles occurred in a ratio of 29:71, a highly significant deviation from the expected ratio of 50:50.

An antigen of a more recently detected blood group system, R, appears to act as a cell-surface receptor for the B subgroup of avian leukosis-sarcoma viruses. The allele R¹ is transmitted simultaneously with the gene b¹, previously established as controlling the early steps of cellular infection by subgroup B viruses. Further studies are being carried out to develop isohemagglutinating reagents for other antigens of the R system and to discover isoantigens that may be acting as receptors for the A and C subgroups of avian leukosis-sarcoma viruses.

**RESUME**

On a montré que la résistance à la maladie de Marek est associée avec l'hérité d'allèles spéciaux du locus du groupe sanguin B qui est très polymorphe chez les poules. Dans des expériences faites dans des conditions naturelles aussi bien que artificielles, un groupe de chercheurs a trouvé que la mortalité chez les poules ayant un certain allèle pourrait être deux fois plus grande que celle des poules ayant un allèle opposé. Par exemple, pendant la couvaison, la mortalité des poulets ayant hérité B¹⁺ ou B²⁻ dans les expériences d'inoculation, a été 29% et 16%, respectivement. Un autre groupe de chercheurs a observé un épuisement différentiel des génotypes du groupe sanguin B chez la progéniture des cogs hétérozygotes aprèrs une éruption sévère de la maladie de Marek — la progéniture
héritant des allèles opposés a eu un rapport de 29:71 — ce qui montre une déviation très importante du rapport de 50:50 auquel on s'attendait.

Un antigène du système de groupe sanguin, R, découvert récemment, semble agir comme récepteur de la surface cellulaire pour le sous-groupe B des virus avian leukosis-sarcoma. L'allèle \( R' \) est transmis simultanément avec le gène \( b' \), qui a été préalablement établi comme l'agent qui contrôle les premières étapes de l'infection cellulaire par les virus du sous-groupe B. On fait maintenant d'autres études pour développer des réactifs iso-hémagglutinants pour d'autres antigènes du système R, et pour découvrir des iso-antigènes qui pourraient agir comme récepteurs pour les sous-groupes A et C des virus avian leukosis-sarcoma.

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**REFERENCES**


