Genetic Susceptibility to and Presence of Endogenous Avian Leukosis Viruses Impose No Significant Impact on Survival Days of Chickens Challenged with Very Virulent Plus Marek’s Disease Virus

Shuang Chang1,2#, Qingmei Xie1,3#, Chong Wang1,3, Mohammad Heidari1, Catherine W. Ernst4, John R. Dunn1 and Huanmin Zhang1,4*

1USDA, Agriculture Research Service, Avian Disease and Oncology Laboratory, USA
2College of Veterinary Medicine, Shandong Agricultural University, China
3College of Animal Science, South China Agricultural University, China
4Department of Animal Science, Michigan State University, USA
#Both contributed equally
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Abstract

Chicks of distinct genotypes at the tumor virus B locus (TVB) in combination with presence or absence of endogenous avian leukosis virus ev21 gene in their genomes were examined for survival day patterns after challenge with very virulent plus Marek’s disease virus (vv+MDV) in three consecutive trials under controlled conditions. The distinct genotypic groups of the challenged birds were TVB*S1/S3 & ev21+/-, TVB*S3/S3 & ev21+/-, TVB*S1/S3 & ev21-/-, and TVB*S3/S3 & ev21-/- with a frequency of approximately one quarter each. Chickens with the genotype TVB*S1/*S3 are genetically susceptible to the subgroup E avian leukosis viruses; and those with the TVB*S3/*S3 genotype are resistant. Marek’s disease (MD) was diagnosed in all birds within 8 weeks post-challenge with a partially attenuated vv+MDV strain (648A passage 40), despite the fact that all chicks challenged were homozygous for MHCB*21 haplotype, which is known to be relatively resistant to MD. No significant difference in survival day patterns was detected between the chicken genotype groups (P > 0.05). The survival day pattern difference was only detected between the challenged and the unchallenged control groups in all three trials (P < 0.001). The findings from this study suggest the genetic nature in resistance or susceptibility to subgroup E avian leukosis viruses in combination with the presence or absence of the endogenous avian leukosis virus ev21 gene in the chicken genome are not capable of altering MD incidence nor distorting survival day patterns in chickens like those White Leghorns used in this study under the specific challenge conditions. If any influence of avian leukosis virus on MD exists, it may be heavily dependent on genetics of chickens and challenge conditions including varied virulence of MDV.

ABBREVIATIONS

ADOL: Avian Disease and Oncology Laboratory; ALV: Avian Leukosis Virus; GA: a strain of MDV; MD: Marek’s Disease; MDV: Marek’s Disease Virus; TVB: Tumor Virus B; vMDV: Virulent MDV; vv+MDV: very virulent plus MDV.

INTRODUCTION

Two of the main classes of tumor viruses that cause neoplasms in poultry are avian leukosis virus (ALV), a retrovirus, and Marek’s disease virus (MDV), an α-herpesvirus [1]. ALV is classified to the Alpharetrovirus genus of the Retroviridae family [1,2]. Six subgroups of ALV are known in chickens, and are referred to as ALV A, B, C, D, E, and J subgroups, which can be distinguished from one another on the basis of different viral envelopes, and subsequently, antigenicity [3,4]. The subgroups A, B, C, D, and J are known as exogenous ALV, whereas the subgroup E is referred to as endogenous ALV based on modes of transmission. Exogenous ALV is transmitted horizontally or congenitally via the eggs. Endogenous ALV is transmitted primarily through chicken germ line although both horizontal and congenital transmissions are also possible [5-8]. The endogenous subgroup E ALV is expressed from one or more endogenous virus (ev) loci in the White Leghorn genome [9-16]. While most of the ev genes are structurally incomplete and only encode defective, noninfectious viral particles, the others, which include ev2, ev11, ev12, ev14,
ev18, ev19, ev20, and ev21, encode infectious virus particles with low or no oncogenicity [13,17-20].

ALV causes a variety of solid tumors including fibrosarcoma, chondroma, haemangiomia, histiocytic sarcoma, mesothelioma, myxoma, nephroblastoma, osteoma, and osteosarcoma [21-23]. The subgroup A and B ALV predominately cause lymphoid leukemia in chickens around four months of age. Subgroups C and D are laboratory strains and are rarely found in the field. Subgroup J ALV predominately causes myeloid leukemia in broilers and also in egg-layer chickens [1,3,4,22,24,25]. Subgroup E is very common, especially in commercial broilers, and is known as the low or non-oncogenic ALV [18]. However, since the success in control of ALV has been achieved through virus eradication schemes that interrupts vertical transmission of ALV from one generation to the next, endogenous ALV and the ev loci, notably those capable of coding for infectious complete virus particles, such as ev21 linked to the K slow-featherling gene, reportedly have detrimental impacts on response to exogenous ALV infection, and on the success of eradication schemes. More detailed information on ALV is described by Payne and others [1,26-31].

ALV infection is mediated by cellular receptors encoded by host genes at loci including tumor virus A (TVA), B (TVB), C (TVC), and J (TVJ) [1,27]. TVB is an autosomal locus encoding cellular receptors to mediate infection of the subgroup B, D, and E ALV [33-37]. Apparently, TVB is relatively more complex, in contrast to the other loci, since it transcribes multiple alleles encoding multiple receptors to facilitate the infection for three of the six major subgroups of ALV. Three alleles at the TVB locus are commonly identified in chicken flocks, which are EV21*TVB*S1, TVB*S3, and TVB*TVB*S1-encoded receptors mediate subgroup B, D, and E ALV infection. TVB*S3-encoded receptors only permit subgroup B and D ALV infection [38]. TVB*R encodes a dysfunctional receptor that blocks ALV B, D, and E subgroups from initiating infection cycles [39].

MDV has been classified into three antigenically related serotypes [40]. Serotype 1 viruses (Gallid herpesvirus 2) are pathogenic, inducing lesions in susceptible chickens. Serotype 2 viruses (Gallid herpesvirus 3) consist of naturally occurring, non-pathogenic MDV strains. Serotype 3 viruses (Meleagrid herpesvirus 1) are the non-pathogenic turkey herpes viruses or HVT. The serotypes 1, 2, and 3 viruses are also referred to as MDV-1, MDV-2, and MDV-3, respectively. Pathogenic MDV causes a lymphoproliferative disease in susceptible chickens characterized by high mortality with or without tumors of the viscera within 8 weeks post infection [41]. Marek's disease (MD) became a problem in the United States in the 1950's as a major disease to control costing the world poultry industry about $2 billion each year [41,42].

It is documented that both herpesviruses and retroviruses are associated with a variety of diseases in mammals and poultry [43-45]. Earlier studies suggested there exists an interaction between ALV and MDV, which contributes to neoplasms in chickens [46-50]. In a field study, a significantly higher level of mortality attributable to lymphoid leukemia was observed in two out of five commercial lines of chickens vaccinated with or contact-exposed to a bivalent MD vaccine, SB-1 plus HVT (MDV-2 and MDV-3) [51]. In a different study, virulent or attenuated MDV moderately enhanced lymphoid leukosis in multiple experimental and one commercial lines of White Leghorns but not in one other experimental line resistant to, or in four other commercial lines susceptible to ALV [41]. MDV reportedly enhanced ALV gene expression and virus production, acting as cofactors in retrovirus replication and pathogenesis in vitro [44]. This study was designed to examine if the presence of endogenous ALV and host susceptibility to endogenous ALV alter the survival day pattern of chickens challenged with a very virulent plus (vv+) strain of MDV (vv+MDV) under a controlled condition.

MATERIALS AND METHODS

Experimental chickens

A total of 116, 144, and 148 F1chicks (Table 1) were reproduced from a cross between the Avian Disease and Oncology Laboratory (ADOL) line 0.44-VB*S1-EV21 (line EV21) males and line 0 females for three consecutive trials, respectively. The line EV21 males possess unique characteristics among the ADOL genetic lines of chickens, which are all heterozygous at both of the ev21 (ev21+/-) and TVB (TVB*S1/*S3) loci. Line EV21 birds are known to produce complete endogenous viral particles at a high expression level and are susceptible to all subgroup ALV (C/0), including subgroup E ALV [18,52]. Line 0 hens are free of ev genes including ev21 (ev21-/-), homozygous at the TVB locus for the TVB*S3 allele (TVB*S3/*S3), and resistant to subgroup E ALV (C/E). Since ev21 in chromosome Z and TVB resides on chromosome 22, alleles at both of the loci obey the law of independent assortment. The 0.44-VB*S1-EV21 males were expected to produce four types of gametes, ev21*+/TVB*S1, ev21*+TVB*S3, ev21*+TVB*S1, and ev21*+TVB*S3, with a frequency of approximately 1/4 each. The line 0 females were expected to produce only one uniform gamete, ev21*+TVB*S3. Therefore, the two lines of chickens were chosen to cross to produce one quarter of chicks expected to be ev21*+/TVB*S1/*S3, one quarter ev21*+/TVB*S3/*S3 (slow feathering birds), one quarter ev21*+/TVB*S1/*S3, one quarter ev21*+TVB*S3/*S3 (rapid feathering birds). All of chicks from the cross were B*21 haplotype homozygous for the major histocompatibility complex.

All chickens used in this study were housed in a BSL-2 experimental facility during each trial. Feed and water were supplied ad libitum. The chickens were observed daily throughout the entire duration of each experiment. The challenge experiments were approved by USDA, Avian Disease and Oncology Laboratory Animal Care and Use Committee (ACUC). The ACUC guidelines established and approved by the ADOL ACUC (April 2005) and the Guide for the care and use of Laboratory Animals by Institute for Laboratory Animal Research (2011) were closely followed throughout the experiments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Trials</th>
<th>Total</th>
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<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>MDV challenged</td>
<td>93</td>
<td>121</td>
</tr>
<tr>
<td>Control</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>116</td>
<td>144</td>
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Locus-specific tests for the presence of ev21

Gene-specific PCR was performed to determine the presence of ev21 for each experimental bird (regardless of feathering status) using DNA samples extracted from blood samples taken on the 23rd day post MDV challenge. The gene-specific primers used in this analysis were Ev21.up, Ev21.dwn, and LTRB. PCR reported by, and PCR product analyses were conducted following the procedures described by Benkel and Tixier-Boichard et al. [13,53].

TVB genotype analysis

All birds were SNP-typed for the TVB genotypes with our previously described Pyrosequencing assays [32] using the same DNA samples that were used for ev27 tests. Briefly, PCR products were generated using the gene-specific primers, TVB303 forward and reverse primer pair [54], of which the TVB303 reverse primer was biotinylated at the 5’ end and high-performance liquid chromatography purified. The PCR products were subsequently SNP-typed with the Pyrosequencing assays using a 16-base forward sequencing primer [32] on a PSQ™ 96MA Pyrosequencing system. The SNP data were analyzed with the PyroMark Q96 ID software version 2.5.8.15 (Qiagen, Maryland, USA).

Cells, viruses and virus challenge

Line 0 primary chicken embryonic fibroblasts were used in preparation of the partially attenuated vv+MDV, 648A passage 40 (648A-P40) [55]. All chickens of the MDV challenged treatment group in each trial were inoculated on day 5 post hatch, with the vv+MDV, 648A-P40, intra-abdominally at a dose of 500 PFU per bird. The chickens of the control groups were not challenged with MDV (see Table 1 for experiment layout for each trial).

Pathological examinations

Chickens that died during or were euthanized at the end of each trial were recorded and examined for gross MD lesions and days survived post-MDV challenge. All experimental birds were pathologically categorized either as MD or normal according to necropsy records. MD refers to those with gross-or histologically confirmed micro-tumors, and/or nerve enlargement(s), or died during the period between 8-day post MDV challenge and before experimental termination. Normal refers to those that survived throughout the challenge experiment period and were free of any MD symptom.

Statistical analyses

The differences in survived day patterns post-MDV challenge between the genetic groups of experimental chickens, defined by the genotypes at both ev21 and TVB loci, were examined by survival analysis and tested with Log-Rank and Wilcoxon statistics. The survival analyses were performed using Statistical Analysis Software JMP version 12 (SAS Institute Inc., NC, USA) [56,57].

RESULTS AND DISCUSSIONS

All infected birds of the challenge trials in this entire study, regardless of TVB genotypes and presence or absence of avian leukosis virus ev21 gene, developed MD within 8 weeks post 648A-P40 inoculation. The 648A-P40 is a higher passage of the vv+MDV 648A stock maintained at ADOL. It is considered partially attenuated due to consecutive in vitro passaging which results in decreased virulence of passaged viruses [55]. Our data has shown 648A-P10 induces over 95% and 100% MD in ADOL line 6, and 7, birds, respectively [58]. In contrast, the 648A-P40 induced no MD in line 6, birds, but still 100% MD in line 7, (Zhang et al., unpublished data), just as did in the F1 birds of line EV21 and line 0 in this study. Table 2 lists the averages of survived days post MDV challenge for each of the challenged genotype groups (ev21+/- & TVB*S1/*S3, ev21+/- & TVB*S3/*S3, ev21-/- & TVB*S1/*S3, ev21-/- & TVB*S3/*S3) and the control groups for all three trials. None of the challenged genotype group differed in survival days from any other challenged group (P> 0.05) but all differed from the corresponding control group in each of the trials (P<0.001). The average survived days post MDV inoculation for the challenged groups of the entire study was 39 ± 0.4 days, which differed significantly from the average (56 ± 0.3 days) of the control groups (P<0.001).

Survival day patterns depict the sequential events of bird loss due to disease progression post exposure to pathogen(s) or inoculation of a known pathogen. Figure 1 gives the survival plots of all four genotypic groups post challenge and the control group for trials 1-3 (plots A, B, C) and the survival plot for all groups of the entire study (plot D). Based on both Log Rank and Wilcoxon statistics, the survival day patterns of the four genotypic groups did not differ from one another (P>0.05) but did from the control group (P<0.001) for each of the three trials, suggesting that neither the TVB genotypes nor the presence or absence of the endogenous avian leukosis ev21 locus altered MD incidence or distorted disease progression post MDV challenge in the experimental White Leghorn birds under the trial conditions.

Endogenous avian leukosis viruses commonly reside in the genome of chickens in most experimental and commercial lines/flocks, especially in broilers [11,14,16]. The ev genes are reportedly associated with production traits [15,59-61], immune response to avian leukosis and reticuloendotheliosis virus infection [62,63], avian leukosis virus shedding [7,8], feathering status [64], and maybe even escalated mortality rate [65] in chickens. Several ev genes were also previously reported in association with genetic resistance to MD in chickens [59]. Reports from more than one laboratory also suggest serotype 2 MDV plays an enhancement role in lymphoid leukosis development in chickens during the 1970s-1990s [41,66-68]. Our latest (unpublished) data showed serotype 2 MDV increased the incidence of spontaneous lymphoid leukosis-lymphomas, as did subgroup E avian leukosis viruses in an ev-free line of chickens. Furthermore, a combination of the serotype 2 MDV and subgroup E avian leukosis virus drastically increased the incidence of spontaneous lymphoid leukosis-like lymphomas in the same line of chickens by several folds (Mays et al., unpublished data). The results of this study, however, showed lack of association between the ev21 gene and MD in chickens, which is not in agreement with the literature [49,59,60]. This incongruity probably resulted from differences of multiple factors between this study and early reported studies, which include genetic backgrounds of chickens, the virulence of challenge viruses, and other challenge conditions. For instance, Kuhnlein et al. [60] used chickens from the directionally selected Cornell strain K

Table 2: Average Survived Days of White Leghorns Distinctly Differed at the TVB Locus and at the Presence of an Endogenous Avian Leukosis Viral Gene post MDV Challenge.

| Genotype† | Trials | 1' | 2' | 3' | Total
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<tr>
<td>TVB*S1/*S3 &amp; ev21-/-</td>
<td>40 ± 1.7b</td>
<td>40 ± 1.4b</td>
<td>37 ± 1.3b</td>
<td>39 ± 0.8b</td>
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<tr>
<td>TVB*S1/*S3 &amp; ev21+/+</td>
<td>43 ± 1.9b</td>
<td>38 ± 1.8b</td>
<td>36 ± 1.3b</td>
<td>38 ± 0.9b</td>
<td></td>
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<tr>
<td>TVB*S3/*S3 &amp; ev21-/-</td>
<td>42 ± 1.7b</td>
<td>39 ± 1.1b</td>
<td>34 ± 1.2b</td>
<td>39 ± 0.8b</td>
<td></td>
</tr>
<tr>
<td>TVB*S3/*S3 &amp; ev21+/+</td>
<td>40 ± 1.3b</td>
<td>39 ± 1.2b</td>
<td>38 ± 2.0b</td>
<td>39 ± 0.9b</td>
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<tr>
<td>Sub-Total</td>
<td>41 ± 0.8b</td>
<td>39 ± 0.7b</td>
<td>36 ± 0.7b</td>
<td>39 ± 0.4b</td>
<td></td>
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<tr>
<td>Control</td>
<td>55 ± 0.0a</td>
<td>54 ± 0.8a</td>
<td>58 ± 0.0a</td>
<td>56 ± 0.3a</td>
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†ev21-/- = endogenous avian leukosis gene ev21 absent; ev21+/+ = ev21 present.
†Average of survived days ± standard error; Averages not sharing a common superscript within a column are significantly different from each other (P<0.001).

and strain S for MD resistance and susceptibility, respectively, in evaluation of differential incidence of endogenous virus genes between the lines of chickens; Aggrey et al. [59] used the same strains of chickens in evaluation of the association between the endogenous virus genes and MD resistance as well as production performance. The study on the interaction between MDV and ALV in vivo by Peters et al. [49] differed from the current study at experimental design including MDV (GA, a vMDV versus 648A-P40, a partially attenuated vv+MDV, [69]) and ALV (subgroup B versus subgroup E) used. Further investigations are deemed necessary to collect additional evidence from experiments to be conducted under varied conditions, such that a clear understanding on the association between endogenous ALV and MD could be gained and a scientific inference could be derived on this subject.

CONCLUSION

In conclusion, three challenge trials were conducted with 342 White Leghorn chickens from a cross between the ADOL line 0.44-VB*S1-EV21 (line EV21) and line 0, in addition to 66
chickens from the same cross used as negative controls for the trials. Despite the fact that all of the chickens were homozygous for MHC B*21 haplotype, MD was diagnosed in every challenged bird within 8 weeks post-challenge using a partially attenuated vv+MDV strain (648A-P40). No significant difference in survival day patterns was detected between the genotype groups, which were either genetically susceptible (TVB^S1/*S3) or resistant (TVB^S3/*S3) to subgroup E avian leukosis viruses and possessing or lacking of the endogenous avian leukosis virus ev21 gene in the genomes of the challenged chickens (P > 0.05). Survival day pattern difference was only detected between the challenged and the unchallenged control groups (P < 0.001) in all three trials. The findings from this study suggest the genetic nature in resistance or susceptibility to subgroup E avian leukosis viruses in combination with the presence or absence of the endogenous avian leukosis virus ev21 gene in the chicken genome are not capable of altering MD incidence nor distorting survival day patterns in chickens like the White Leghorns under the specific challenge conditions used in this study. Our findings support the conclusion that the influence of avian leukosis virus on MD may be heavily dependent on the genetics of chickens and challenge conditions, primarily including MDV of varied virulence. Further studies are warranted to elucidate the complex relationship among host genetics background, endogenous avian leukosis viruses, and MDV, which, together, would result in prevention or promotion of tumorigenesis.

REFERENCES

