THE ENDOGENOUS VIRAL GENE *ALVE6* DOES NOT AFFECT RESISTANCE TO MAREK'S DISEASE IN FAYOUMI CHICKENS

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SUMMARY

A sample of 103 Fayoumi chicks and 50 White Leghorn chicks were challenged at 18 days of age by Marek's disease virus. The Fayoumi strain was segregating for endogenous viral loci, including *ALVE6* which could have unfavorable effects on immune response. Incidence of the disease was much lower in the Fayoumi (43%) as compared to the White Leghorn control (76%) but was not affected by *ALVE6*. Affected Fayoumi chicks died 30 days later than the controls, whatever their genotype for *ALVE6*.
Key words : chicken, endogenous viral gene, Marek's disease, Fayoumi breed.

INTRODUCTION

The Fayoumi breed from Egypt is known to resist to unfavorable environmental conditions, namely high temperature and exposure to pathogens (Hossary and Galal, 1994). The unique origin of this breed makes it also a valuable animal material for studies of genetic diversity. For all these reasons, an experimental population was set up in 1978 at the Lab. of Génétique Factorielle from a sample of fertile eggs obtained in Egypt (Mérat and Bordas, 1982).

This Fayoumi population was included in a survey of the polymorphism of endogenous viral genes (Tixier-Boichard *et al.*, 1994). The chicken is known to harbor a variety of retroviral insertions in its genome, the most well-known family being the E subgroup of avian leukosis viruses, as reviewed by Crittenden (1991). The Fayoumi breed appeared to carry several *ALVE* loci at a moderate frequency, which showed very little homology with endogenous proviruses previously characterized in White Leghorns. One remarkable exception was the presence of *ALVE6*, which has been found also in many other chicken breeds. This locus has a deleted viral structure but leads to the production of the envelope protein. Because of this expression, *ALVE6* increases the susceptibility to infection by exogenous leukosis viruses (Robinson *et al.*, 1981). Unfavorable effects of *ALVE6* have been suggested by frequency studies conducted on lines selected either on immune response (Lamont *et al.*, 1992) or on resistance to Marek's disease (Kühnlein *et al*, 1989). A decreased frequency of *ALVE6* was found in two White Leghorn lines selected for resistance to Marek's disease (Kühnlein *et al*, 1989).

The objective of the present study was to investigate the effect of *ALVE* loci, and in particular of *ALVE*6, on Marek's disease, within the Fayoumi line.

MATERIALS and METHODS

A set of 103 one-day old female chicks was produced from 6 sires and 30 dams of the randombred Fayoumi experimental line kept in Jouy-en-Josas. Parents had been vaccinated against Marek's disease (Rispens strain) but their offsprings were not and were immediately transferred to the specific-pathogen free (SPF) with filtered air pressure facility of CNEVA in Ploufragan. A set of 25 male and 25 female one-day old SPF chicks was obtained from a closed randombred White Leghorn (WL) strain kept in Ploufragan, to serve as a control for the challenge. The Ploufragan VMA70S1 strain of Marek's disease virus was isolated in 1969 and prepared on primary cultures of chick embryo fibroblasts from the WL SPF strain. A suspension of titrated virus corresponding to 2000 PFU in 0.4 ml was inoculated intra-peritoneally at 18 days of age to the Fayoumi and Leghorn chicks. From day 18 on, the animal facility was maintained under negative pressure. The effects of the inoculation were monitored for 20 weeks. Dead birds were recorded daily and necropsied. Macroscopical lesions typical for Marek's disease were investigated but no histological analysis was performed. Lesions were scored according to their location, either neural or visceral, and to their aspect, simple hypertrophy or tumors. Birds surviving after 20 weeks were killed and necropsied.

On the day of viral inoculation, 1 ml of blood of each Fayoumi chick was sampled on EDTA. Genomic DNA was extracted from 50 µl blood after hemolysis, incubation with proteinase K and precipitation with acetone/dimethylformamide 95/5. The same procedure was applied to a subset of 10 White Leghorn controls and to the Fayoumi parents. *ALVE* loci were identified by restriction fragment length polymorphism obtained after digestion with *Sac*I or *Bam*HI enzymes and hybridization with the RAV-2 probe as described previously (Tixier-Boichard *et al.*, 1994). The *ALVE*6 locus was identified by its characteristic *Sac*I and *Bam*HI junction fragments (25 kbp and 4.2 kbp) and further confirmed by the study of representative DNA samples with a locus-specific PCR diagnostic test provided by B. Benkel (unpublished data).

The type of lesions and the occurrence of death were discrete traits that were analysed by the CATMOD procedure of the Statistical Analysis System (SAS). The age of death was continously and normally distributed among Fayoumi chickens that died from Marek's disease before the end of the experiment. An analysis of variance was performed under a linear model, according to the GLM procedure of SAS. Analysis was performed in two steps : observations from both strains were analysed to estimate the effects of strain and sex ; observations from the Fayoumi strain were analysed to estimate the effects of sire, of *ALVE*6 carrier status and of number of *ALVE* loci per bird.

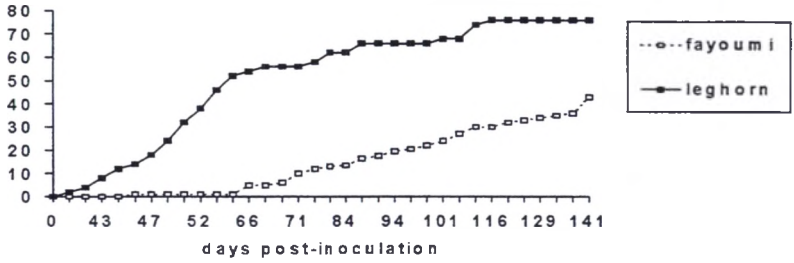

Figure 1. Cumulated percentage of dead chicks and carriers of lesions following an inoculation of Marek's disease virus at 18 days of age.

RESULTS

All the chicks that died before the end of experiment exhibited specific lesions from Marek's disease, thus excluding the effect of an interfering disease. Among the 103 Fayoumi chickens, 6 birds were males and appeared to be sexing errors. They were kept for the analysis because no sex effect was observed in the control WL SPF sample. The proportion of dead birds was significantly lower in the Fayoumi as compared to the SPF WL control (Table 1, Figure 1). The Fayoumi chicks died significantly later than the White Leghorns. The lesions were predominantly visceral in Fayoumi whereas they were predominantly neural in White Leghorns. Tumor development affected mainly liver and spleen and to a lesser extent kidney.

Table 1. Response to an inoculation of Marek's disease virus at 18 days of age in Fayoumi and White Leghorn chickens.

line	sample size	% dead	% killed with lesions	incidence (%)	age at death (days post-infection)	% of visceral lesions	% of neural lesions
Fayoumi	103	36	7	43	93	84	32
WL SPF	50	76	0	76	63	45	71
line effect	-	$p<0.0001$	not tested	$p<0.0001$	$p<0.0001$	$p<0.003$	$p<0.001$

The Fayoumi sample showed segregation for 11 *ALVE* loci, including *ALVE*6. The number of loci in each individual varied from 0 to 5 with an average of 2. Only 5 loci had a frequency above 20 %. Since *ALVE*6 was the most frequent (52%) and was segregating in the 6 sire families, its presence was considered as a fixed effect cross-classified with the sire effect. In

two families, however, *ALVE*6 was not found in the sire but was transmitted by several dams. In the case where both sire and dam could transmit *ALVE*6, heterozygous and homozygous progeny for *ALVE*6 could not be distinguished, because both RFLP and PCR test provide only a presence or absence result. The sample of WL chicks studied by RFLP showed the presence of 5 *ALVE* loci, including *ALVE*6 that was found in 4 out of 10 tested chicks.

Within the Fayoumi line, the age at death was not significantly affected by the sire family, the number of *ALVE* loci per bird and the presence of *ALVE*6. The model explained only 24% of the total variance. Incidence of Marek's disease (death or lesions at 20 weeks) was 35% in carriers of *ALVE*6 and 44% in non carriers, which was not a significant difference. The sire family had no significant effect on disease incidence but one sire appeared to have only 13% affected progeny whereas incidence ranged from 30 to 50% in the other families.

DISCUSSION

The challenge was done at 18 days of age because the effect of *ALVE*6 on Marek's disease resistance had been suggested when challenge was done at 3 weeks of age (Kühnlein *et al.*, 1989). At that age, maternal antibodies should be at a low level and immune response of the chick is likely to be functional, so that differences in susceptibility to disease might be mediated by differences in immune function. The effect suggested for *ALVE*6 in White Leghorns was not observed in Fayoumi. This could be due to the effect of the background genome or to the limited sample size which prevented the identification of small or moderate effects. Furthermore, the mechanism of interaction of *ALVE*6 with Marek's disease infection is not quite understood in White Leghorns, because immune response parameters measured in infected or uninfected chickens do not seem to be influenced by *ALVE*6 (Lessard *et al.*, 1994). The present study confirmed the resistance of Fayoumi chickens to Marek's disease. Mechanisms of this resistance are not identified and influence of major histocompatibility complex should be investigated in the future.

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