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Observation on Viral Variation and Vaccine Development in Marek's Disease in China

[1]Zhenhua Gong, [2]Lijuan Zhang, [1]Ping Lu, [2]Yonghao Hu, [1]Liping Wang and [1]Weihua Li
[1]China Animal Healthy and Epidemic Center, 266032 Qingdao, China
[2]Gansu Agriculture University, 730070 Lanzhou, China

Abstract: Marek's Disease (MD) is one of the main infectious diseases that threaten the healthy development of the Chinese poultry industry. The evolution of epidemic strains of the MD Virus (MDV) in China is different from the evolution of strains overseas. The *meq* gene sequence of pathogenic strains is different from that of the attenuated vaccine strain (MDV-1). The *meq* and *L-meq* gene sequences of the MDV in the lymphoid tissue of chickens in latency period are homologous. The *meq* gene was only found in the lymphoid tissue of chickens during the onset period.

Key words: Marek's disease, viral variation, vaccine, epidemic, evolution

INTRODUCTION

Marek's Disease (MD) is one of the main infectious diseases that threaten the Chinese poultry industry. Since, it was reported in the 1970s, MD has become the major epidemic disease that affects the healthy development of the poultry industry. In recent years, biological character changes were found in MD Virus (MDV) isolated strains such as genetic variation, enhanced virulence and breakthrough of the MD routine immunization. Great concern should be focused on the emergence of variant strains even though in China at present, the improved vaccine producing technology was developed and the widely adopted MD immunization strategy had played a significant role on controlling the disease.

GENERAL

Epidemic history: MD was sporadic in early years in China. In 1973, MD was reported in chickens from different regions in China such as Beijing, Shanghai, Harbin, Dalian and Qingdao where it caused serious economic losses. Native chickens are less sensitive to the disease than exotic chickens. The infected chickens showed mild neurologic symptoms or visceral lymphomas. In the mid-1980s, an isolated virulent strain reportedly caused severe MD in chicken immunized with the Herpes Virus of Turkey (HVT) vaccine and it caused disease in chickens with intrinsic genetic resistance to this disease. In the 1990s, a virulence-enhanced MDV mutant emerged in China that overcomes the immunity conferred by the MD HVT vaccine by the HVT + MD-1 bivalent vaccine causing visceral lymphomas in chickens and early non-neoplastic death in broilers.

Current epidemic status: Since the 1970s, the HVT vaccine has been used in China to control the MD epizootic situation. Significant results were achieved in the early years. However, in recent years, a vv + MDV mutant emerged which resulted in immune failure among chickens in some regions, especially in Hebei, Henan, Shanghai, Jiangsu, Guangdong and Guangxi which are the main chicken producing provinces in China. The vv + MDV mutant had been a major threat to hazard of the poultry industry in these regions since the 1990s. The morbidity rate of MD among flocks remained at 30%, even after immunization with HVT + MD-1 bivalent vaccine. The mortality rate of the neural type of MD ranged from 1-3% whereas that for the visceral-type ranged from 25-30% (Zhou *et al*., 2003). Exotic broiler chicken and egg chicken as well as quail and pheasant were infected with MDV. MD was also found in two native chicken breeds, the Barred Plymouth Rock and the three Yellow chickens which were earlier considered resistant to MD (Yu *et al*., 2009; Zuo *et al*., 2007).

Clinical symptoms: Chickens 2-5 months old were usually infected with MDV but 50-70 days old chickens are even more commonly affected. Even 45 days old broiler chickens and 180-200 days old egg chickens are susceptible to infection. Boiler chickens infected with MD

Corresponding Author: Zhenhua Gong, China Animal Healthy and Epidemic Center, 266032 Qingdao, China

usually develop visceral symptoms. Chicken develop both visceral and neural symptoms although, neural symptoms generally occur in 3-4 months old chickens.

MDV VARIATION CHARACTERISTICS

The field MDVs from China formed an independent cluster: Homology analysis of *Meq*, *pp38* and *vIL-8* genes of 18 field MDV strains isolated in Sichuan province, China showed that the deduced amino acid sequences of these three genes exhibit 95.0-98.8, 99.3-100 and 97.0-98.5% homology, respectively with these of other reference strains published in GenBank (Tian *et al*., 2011). Alignment analysis of the nucleotide and deduced amino acid sequences of 27 isolates from China showed that four amino acid mutations in *Meq* gene and two amino acid mutations in *vIL-8* gene displayed perfect regularity. As for *Meq* gene, it was found that some amino acid mutations at position 80, 115, 139 and 176 in most isolates from China displayed perfect regularity. Mutation at position 80 (aspartate→tyrosine), 115 (valine→alanine), 139 (threonine→alanine) and 176 (proline→arginine) accounted for 96.3% (26/27), 100% (27/27), 90.0% (24/27) and 96.3% (26/27) of all field MDVs from China, respectively. The mutation in position 80,115, 139, 176 of Meq protein could be used as virulent genetic characteristics of the circulating MDVs MDV in China. As for *vIL-8* gene, the nucleotide mutation at position 11 (T→C) and 92 (A→G) were common in the18 isolates, resulting in two amino acid mutations at position 4 (leucine→serine) and 31 (aspartate→glycine) of MDVs from China. Phylogenetic tree, based on the Meq and vIL-8 amino acid sequences, revealed that the field MDVs from China formed an independent cluster while vaccine strains, mild virulent MDVs and virulent MDVs from USA formed another cluster. The amino acid mutation in the meq protein of almost all the Chinese isolates was characterized as a unique two-point mutation that had a certain correlation with the virulence of MDV and was different from that of the foreign strain (Shi, 2009; Xu, 2011).

Differences between the *meq* genes of pathogenic and attenuated MDV strains: Sequence analysis of the MDV *meq* gene showed that the pathogenic isolated strains 648A, G2, N, 0093, 0095, 0297 and 0304 had conserved nucleotide and amino acid sequences and that the isolated strains were homologous. Unlike the CVI988/Rispens and 814 vaccine strains, 2 genomic repeat regions containing 15 amino acid residues (EELCAQLCSTPPPPI) and the 4 genomic repeat regions containing 6 amino acid residues (PPICTP) were first discovered in these pathogenic strains. The repeats were both located in the C-terminal transcription activation domain of the meq protein. The *meq* gene expression product was detected in Chicken Embryonic Fibroblasts (CEF) and in natural tumor cells infected with MDV using anti-meq monoclonal antibodies but it was not detected in cells infected with the CVI988/Rispens strain (Wei *et al*., 2003).

Difference between meq and L-meq: In Jilin University, gradient Polymerase Chain Reaction (PCR) was used to obtain the *meq* and *L-meq* genes of MDV during its latency period. Sequence analysis of the PCR product showed that the *meq* and the *L-meq* genes were homologous. However, only the *meq* gene was found in the lymphatic tissue of infected chickens when the same method was used. To understand why the *L-meq* gene was not detected in the chickens during the onset period, the domains of the amino acid sequence of the *L-meq* and *meq* genes were sequenced. The results show that the *L-meq* gene had nine Proline-Rich Repeat (PRR) regions whereas the *meq* gene had six PRR regions. Therefore, the PRR region may have affected the transcription and activation of the viral gene, thereby controlling viral DNA replication. This finding indicates that the *L-meq* gene is necessary for maintaining the latency (Pan *et al*., 2007).

VACCINE RESEARCH

814 strain: The 814 strain is naturally MDV-1 attenuated strain. It is used as vaccine to control MD for a long time in China. In 1981, a naturally attenuated MD strain was isolated by Liu (2004) from a clinically asymptomatic chicken at a farm in Harbin Suburb, Heilongjiang province. After the isolated natural attenuated virus was passaged on chick embryonic skin cells which became the MDV-1 814 vaccine strain. The sequence analysis showed that the complete DNA sequence of the 814 strain consists of 172, 541 bp. It has overall gene organization identical to the MDV-1 strains (Zhang *et al*., 2012). Phylogenetically, the genome of vaccine strain 814 was more similar to CVI988 strain. Comparative analysis among the 814 strain, virulent strains and CVI988 strain showed that a 177 bp insertion was identified in the overlapping genes encoding the Meq RLORF6 and 23 kDa proteins of strain 814, the same with vaccine strain CVI988; 69 bp deletion in the gene encoding RLORF12 of strain 814 was found. The deletion was located in the Origin of replication site (Ori) of MDV. A deletion of 510 bp in the UL36 protein which was the largest structural protein within the tegument of virions was also identified. A total of 24 Single Nucleotide Polymorphisms (SNPs) common to both

814 and CVI988 and not occurring in virulent strains (Liu, 2004). Laboratory tests proved that the protective efficacy of the 814 vaccine is 89%, higher than the CVI988 vaccine (79%) (Ma *et al*., 2009). The 814 vaccine was proven to reduce chicken mortality from 7% by HVT prevention to 2%. Maternal antibodies did not significantly influence the immunity conferred by the 814 vaccine strain. When the 814 strain vaccine was used to immunize chickens with maternal antibodies but no HVT antibodies, the average of protective efficacy was 89% whereas the immunity provided only by HVT was 60.5%. At 8 days after inoculation with the 814 strain vaccine, the tested chickens exhibited strong immunity against MD (Zhang *et al*., 2009).

Z4 strain: The Z4 strain was an MD serum II vaccine strain isolated in China (Huang *et al*., 1988) from Jiangsu Agricultural College, isolated the virus from a native chicken in a suburb of Yangzhou city. The Z4 vaccine strain was grown in CEF that produced a plaque morphology characterized by a large round syncytium. Inoculation of the strain into specific pathogen free chickens did not cause any clinical symptoms or pathologic changes of MD. Protection experiments showed that the chickens inoculated with the Z4 strain were resistant to virulent MDV strains. The Z4 attenuated strain combined with HVT vaccine provided complete immunity against virulent strains.

Recombinant vaccine: In China, a recombinant MD vaccine was developed by expressing the structural proteins that conferred partial protection *in vitro* experiments. However, the potency of the recombinant vaccine is not as effective as the conventional vaccine (Chen *et al*., 2002) constructed a recombinant virus that expressed the *MDV gL* gene using a Chinese strain of the Fowlpox Virus (FPV) as a vector but the expressed protein did not produce the desired protective effect. In 2003, Ding *et al*. (2003) inserted the *MDV gB* gene into the eukaryotic expression vector pcDNA3 and transfected the recombinant DNA into COS-7 cells *in vitro*. Indirect immunofluorescence staining confirmed that the COS-7 cells transfected with pcDNA3-gB expressed glycoprotein B. The *pcDNA3-gB* gene was injected intramuscularly into the AA broiler chicken which induced an immunoprotection index of 72.2%. Hu (2001) expressed the CVI988/Ripens *gB* gene using group fowlpox virus as a vector. The expressed gB protein was combined with HVT to produce the HVT + rFPV-gB/R bivalent and a freeze-dried vaccine was obtained. The potency experiment showed that the immunoprotective effect of the HVT + rFPV-gB/R bivalent freeze-dried vaccine is superior to that of the HVT freeze-dried vaccine against virulent IBDV Md5 and RBIB attack and it approximated the protective level of CVI988/Ripens.

CONCLUSION

Studies on live and genetically engineered vaccines for Marek's disease were carried out in China. Two MD vaccine strains were developed of which the 814 and Z4 strains conferred immunity against virulent MDV strains in chickens.

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