ABSTRACT

IDENTIFYING THE GENETIC BASIS OF ATTENUATION IN MAREK'S DISEASE VIRUS VIA EXPERIMENTAL EVOLUTION

By

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Marek's disease virus (MDV), an oncogenic alphaherpesvirus of chickens, causes up to $2 billion in loses a year due to Marek's disease (MD). Therefore control of this economically important disease is critical. The primary method to control MD is vaccination. Attenuated, or weakened, strains of MDV have been generated via repeated in vitro serial passage to generate avirulent MDV strains that have been used as successful MD vaccines. Despite introduction of several vaccines since the 1970's, more virulent strains of MDV have evolved to break vaccinal protection. Therefore, development of new MD vaccines is necessary. To address this concern, we sought to better understand the molecular basis of attenuation in MDV to provide information that may assist in the rationale design of MD vaccines.

Three attenuated replicates of a virulent MDV were serially passed in vitro for over 100 passages. DNA and RNA from attenuated viruses were deep sequenced using Illumina nextgeneration sequencers to identify changes in DNA sequence or expression following attenuation.

Top candidate mutations identified via sequencing were used to generate seven recombinant viruses using red-mediated recombineering for mutations within UL42, UL46, UL5, two involving LORF2 and two mutations within ICP4. These recombinant viruses were tested in vivo to determine the impact of these mutations on MD incidence, in vivo replication and horizontal transmission. Point mutations within UL42, UL46, LORF2-Promoter and ICP4 did not cause

observable phenotypic changes compared to the parental virus. A single point mutation within LORF2-Intron and a double mutant involving ICP4 both resulting in 100% MD in challenged birds but failed to transmit horizontally to uninfected contact birds. Finally, a point mutation within UL5 reduced MD incidence by over 90%, significantly reduced in vivo replication, and eliminated horizontal transmission.

Further characterization of this UL5 point mutation determined that it increased in vitro replication in growth curves, yet head-to-head competition of the Mut UL5 virus versus parental virus showed the parental virus outcompeted the mutant virus. Furthermore, serial passage of Mut UL5 in vivo did not result in increased in MD incidence, in vivo replication or result in reversion or compensatory mutations to UL5 after passage through birds. Trials testing vaccinal protection of the Mut UL5 virus showed the virus provided partial protection against challenge with virulent MDV, yet did not exceed protection achieved through use of traditional vaccines. Therefore, use of this point mutation in combination with other candidate mutations was tested. Addition of the UL5 mutation with ΔMeq, a candidate vaccine with high protection and replication but also induces bursal-thymic atrophy (BTA), resulted in a recombinant virus that replicated at low levels and did not cause BTA, yet reduced levels of vaccinal protection, indicating an intricate relationship between replication levels, BTA and vaccinal protection. This study shows that a variety of genes are mutated during attenuation, and particularly mutations within DNA replication genes, such as UL5, appear to play an important role in attenuation. We also determined that experimental evolution is a process that not only can identify mutations involved in attenuation, but also offer protection as a vaccine to provide information for further development of MD vaccines.