ABSTRACT

CHARACTERIZING CHICKEN STEM CELL ANTIGEN 2, A PUTATIVE MAREK'S DISEASE RESISTANT GENE

By

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Marek's disease virus (MDV) is an alpha-herpesvirus that is the causative agent of Marek's disease (MD), a lymphomatous disease of chicken. MD is a serious chronic disease of concern to the poultry industry worldwide, although it has been controlled through the use of vaccination since the 1970's. MD vaccines prevent the formation of lymphomas but are not sterilizing and do not prevent MDV infection or replication in the bird. Consequently, the observed increase in MDV virulence over the years may be an undesirable response to the widespread use of MD vaccines. With increases in MDV virulence expected in the future and the most effective MD vaccine (Rispens) already in use, additional approaches such as genetic resistance to control MD are needed to augment vaccinal control of MD. The overall purpose of this project is to characterize stem cell antigen 2 (SCA2), the product of the putative MD resistant gene, and its role in MDV biology

To analyze the biological properties of chicken SCA2, SCA2 protein was expressed and purified in E. coli, and a polyclonal antibody was developed. Utilizing this anti-SCA2 antibody, SCA2 was identified to be a 13 kDa cell surface protein anchored by a GPI moiety as is the case for most other Ly6 family members. In vivo studies showed unique SCA2 expression pattern in bursal cortical medullary epithelial cells (CMEC), which are surrounded by MHC class II presenting cells. Expression profiles of

bursal cells induced by contact with SCA2-expressing cells in vitro demonstrated downregulation of numerous genes that are involved in the B cell receptor and immune response signaling pathways. These data suggest that SCA2 plays a role in regulating chicken B lymphocytes.

The viral US 10 protein was previously demonstrated to interact with SCA2 in an E. coli two hybrid screen followed by confirmation using an in vitro binding assay. To analyze the functional interaction of SCA2 and US 10 for MDV growth properties, two recombinant MDVs were developed in which viral US 10 gene was fused to or replaced with an enhanced green fluorescent protein (EGFP) coding region. Over-expressing SCA2 impairs both MDV plaque size and the percent of fibroblasts infected in vitro but this effect is dependent on the presence of US 10. We conclude that MDV US 10 is both sufficient and required for this growth impairment via association with SCA2.

A missense point mutation in MDV UL41 gene was found out when cloning the viral genome into bacterial artificial chromosome (BAC). Monitoring the frequency of each SNP by pyrosequencing during virus passages determined the ratio of each viral genome in a single monolayer. This point mutation in UL41 gene enhanced the viral fitness in the competitive growth assay in vitro, but abolished the virion host shutoff (vhs) activity of UL41. This result suggests that the enhanced fitness in vitro for MDV with inactive vhs was due to reduced degradation of viral transcripts.