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Efficacy of various Marek's disease vaccines protocols for prevention of Marek's disease virus-induced immunosuppression

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ABSTRACT

Marek's disease virus (MDV) induces tumors and severe immunosuppression in chickens. MDV-induced immunosuppression (MDV-IS) is very complex and difficult to study. In particular, the late MDV-IS (late-MDV-IS) is of great concern since it can occur in the absence of lymphoid organ atrophy or gross tumors. We have recently developed a model to reproduce late-MDV-IS under laboratory conditions. This model measures MDV-IS indirectly by assessing the effect of MDV infection on the efficacy of infectious laryngotracheitis (ILT) vaccination; hence the name late-MDV-IS ILT model. In this study, we have used the late-MDV-IS ILT model to evaluate if MD vaccination can protect against late-MDV-IS. One experiment was conducted to determine whether serotype 1 MD vaccines (CVI988 and Md5ΔMEQ) could induce late-MDV-IS by themselves. Three additional experiments were conducted to evaluate efficacy of different MD vaccines (HVT, HVT+SB-1, CVI988, and Md5ΔMEQ) and different vaccine protocols (day-old vaccination, in ovo vaccination, and double vaccination) against late-MDV-IS. Our results show that none of the currently used vaccine protocols (HVT, HVT+SB-1, or CVI988 administered at day of age, in ovo, or in double vaccination protocols) protected against late-MDV-IS induced by vv+MDV strains 648A and 686. Experimental vaccine Md5ΔMEQ administered subcutaneously at one day of age was the only vaccine protocol that significantly reduced late-MDV-IS induced by vv+MDV strain 686. This study demonstrates that currently used vaccine protocols confer high levels of protection against MDV-induced tumors (protection index = 100), but do not protect against late-MDV-IS; thus, commercial poultry flocks could suffer late-MDV-IS even in complete absence of tumors. Our results suggest that MDV-IS might not be related to the development of tumors and novel control methods are needed. Further evaluation of the experimental vaccine Md5ΔMEQ might shed light on protective mechanisms against late-MDV-IS.

1. Introduction

Marek's disease (MD) is a lymphoproliferative disease of chickens induced by an alpha-herpesvirus known as Marek's disease virus (MDV) [1]. MDV belongs to the genus *Mardivirus* that includes three species: *Gallid herpesvirus* 2 (serotype 1 MDV), *Gallid herpesvirus 3* (serotype 2 MDV) and *Meleagrid herpesvirus 1* (serotype 3 MDV and also known as herpesvirus of turkeys or HVT). Serotypes 2 and 3 are non-oncogenic viruses isolated in chickens and turkey, respectively. Both of these replicate in chickens and can be used as vaccines [2,3]. Serotype 1 MDV includes chicken viruses that are oncogenic. Based on virulence, serotype 1 MDVs are divided into several pathotypes: mild (m), virulent (v), very virulent (vv) and very virulent plus (vv+) [4,5].

In the absence of vaccination, MD is a devastating disease for the poultry industry [6,7]. MD vaccines were first introduced in 1968 [8]. Current MD vaccines include strains of all three serotypes: Serotype 3 strains, combination of serotypes 2 (SB-1, 301B) and 3 strains; and serotype 1 strain CVI988, which is the most effective vaccine currently used against vv+MDV strains [2,3,9–14]. MD vaccines have been very successful in controlling MDV-induced tumors. However, in spite of the success of MD vaccines, MD is still a problem for the poultry industry due to the continuous evolution of MDV towards more virulence. Newly emergent MDV strains are able to break vaccine immunity and also they induce a variety of syndromes other than tumors, among

Abbreviations: MD, Marek's disease; MDV, Marek's disease virus; MDV-IS, Marek's disease virus-induced immunosuppression; ILT, infectious laryngotracheitis; ILTV, infectious laryngotracheitis virus; PFU, plaque forming units; CEO, chicken embryo origin; CEF, chicken embryo fibroblasts; CKC, chicken kidney cells.

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which MDV-induced-immunosuppression (MDV-IS) is the one of greatest concern [15,16].

Control of newly emergent, highly virulent MDV strains has required the use of various vaccination strategies such as in ovo vaccination, protective synergism, revaccination, adjuvants, and recombinant vaccines. In ovo vaccination is used in broilers and broiler breeders in the USA and its use is becoming more popular in other countries. MD vaccine is injected into embryos at 18 days of embryonation by the amniotic route. In ovo vaccination allows administration of vaccines three days before chickens can get exposed to MDV, reduces labor, and hastens the development of the chicken immune system [17,18]. Furthermore, in ovo administration of MD vaccines results in greater protection against MDV-induced tumors than vaccination at day of age [19–21]. Vaccine efficacy can also be enhanced by combining serotypes 2 and 3 strains in the same vaccine (protective synergism) as the combination provide better protection than individual vaccine used alone [22,23]. Revaccination can also increase vaccination efficacy [24–26]. Deletion of oncogene meq from vvMDV Md5 (Md5ΔMEQ) strain resulted in a vaccine that provide better protection than CVI988 when challenged against vv+ strains at day of age [27–29]. However, this strain induces severe lymphoid organ atrophy in maternal antibody negative chickens and cannot be licensed under current regulations [27].

MD vaccines protect against the development of tumors, transient paralysis, lymphodegenerative syndromes and lymphoid organ atrophy, and arteriosclerosis. However, they do not protect against superinfection and transmission of MDV. Preliminary data suggest that one-day-old vaccination might not protect against late-MDV-IS [30]. MDV-IS can be divided into two phases, early-MDV-IS associated with early cytolytic infection of lymphoid organs and late-MDV-IS that occur during the establishment of latency and tumor development [31]. Early-MDV-IS is controlled by maternal antibodies against MDV and vaccination and it is not considered to have any relevance under commercial conditions [32,33]. Late-MDV-IS could occur in commercial flocks [30] and is a threat to the poultry industry. In a previous work, we have developed a model to study late-MDV-IS under laboratory conditions. This model indirectly evaluates late-MDV-IS by assessing the effect of vv+MDV infection at day of age on the efficacy of infectious laryngotracheitis (ILT) vaccination at 15 days of age followed by challenge exposure with infectious laryngotracheitis virus (ILTV) at 30 days of age. Our studies have shown that late-MDV-IS can happen in commercial chickens bearing maternal antibody against MDV that did not have lymphoid organ atrophy or gross tumors. Late-MDV-IS markedly decreased the efficacy of ILT vaccination [30] and could affect the efficacy of vaccination programs against other diseases as well. It is unknown if MD vaccination protocols that confer high protection against the development of tumors also could protect against late-MDV-IS. Moreover, it is unknown if serotype 1 MD vaccines that induces lymphoid organ atrophy in maternal antibody chickens (i.e. Md5ΔMEQ) can induce late-MDV-IS in the ILT model.

The objectives of this study were to (1) determine if serotype 1 MD vaccines can induce late-MDV-IS and (2) evaluate if MD vaccination protocols that protect against MDV-induced tumors also protect against late-MDV-IS.

2. Materials and methods

2.1. Chickens

Specific-pathogen-free SPAFAS chickens (Charles River SPAFAS, N Franklin, CT) were used as MDV shedders. Female commercial meat type chickens (grandparents) were used as experimental chickens. Experimental chickens came from dams that were vaccinated with vaccines of the three serotypes (HVT, SB-1, and CVI988), therefore they were considered to have maternal antibodies against the three serotypes.

2.2. Viruses and vaccines

Serotype 1 MDV strains, 648A (vv+) at passage 12 in chicken embryo fibroblast (CEF) and 686 (vv+) at passage 10 in duck embryo fibroblast (DEF) with one additional passage in chicken kidney cells (CKC) were used as MDV challenge [5]. Commercial strains HVT [3,11], bivalent HVT-SB1 [2,13,23,34], and CVI988 [9] from two different manufacturers (A and B) were used for vaccine trial. Experimental vaccine, Md5ΔMEQ (passage 5 in CEF) derived from a bacterial artificial chromosome (BAC) was also used [35]. ILT vaccine of chicken embryo origin (CEO) (Merial Select, Inc., Gainesville, GA, USA) and virulent ILTV strain Illinois-N71851 [36] were used.

2.3. qPCR and qRT-PCR

DNA was extracted from feather pulp and spleen using Archive-Pure DNA tissue kit (5-Prime Inc, Gaithersburg, Maryland, USA) and RNA was extracted from spleen using the Perfect Pure RNA tissue kit (5-Prime Inc, Gaithersburg, Maryland, USA) following manufacturer's recommendations. DNA samples were amplified with primers specific for viral genes gB and meq, and for the chicken glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. Transcription of MDV ICP4, pp38, gB and Meq genes as well as housekeeping 28S rRNA was evaluated by qRT-PCR. The sequence for the respective forward and reverse primers are listed in Table 2. Amplifications were done using an Mx3005 (Stratagene, La Jolla, CA) in a 25-ul PCR reactions as reported [37,38].

2.4. Analysis of microRNA expression by qPCR

First strand complementary DNA (cDNA) was synthesized from 1 μg of polyadenylated total RNA from each spleen sample using a miRNA 1st strand cDNA synthesis kit (Agilent, Santa Clara, CA) following the manufacturer's instructions. For miRNA qPCR, each reaction contained 1 μl of cDNA and 24 μl High-specificity miRNA QPCR Core Reagent Kit Master Mix (Agilent Technologies, Santa Clara, CA, USA). PCR was performed using the following conditions: 95 °C for 15 min followed by 40 cycles of [95 °C 10 s and 60 °C 20 s] using Mx3005 Stratagene (Stratagene, La Jolla, California, USA) thermocycler. Expression of MDV miRNA mdv1-miR-M4-5p and chicken snoU83B was conducted using forward primers previously reported and they are listed in Table 2 [39].

2.5. Serology

Antibody titers against ILTV were measured using the ProFLOK® Fowl Laryngotracheitis Virus Antibody Test Kit (Synbiotic Corporation, Kansas City, MO) following manufacturer's recommendations.

2.6. Experimental design

Animal experiments were conducted following the guidance and under approval of North Carolina State University Institutional Animal Care & Use Committee. Three experiments were conducted using the late-MDV-IS ILT model as reported [30]. Briefly, shedder chickens were infected subcutaneously at day of age with 500 PFU of vv+MDV strains 648A (experiments 1 and 2) or 686 (experiment 3) and maintained in isolation for 15 days prior to beginning of experiment. They were used as a source of MDV infection by housing them commingled with 1-day-old experimental chickens.