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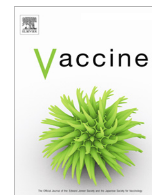

Effects of different promoters on the protective efficacy of recombinant Marek's disease virus type 1 expressing the VP2 gene of infectious bursal disease virus

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A B S T R A C T

The vaccine efficacy of recombinant viruses can be influenced by many factors. Accordingly, the activity of promoters has been one of the major factors affecting the antigen expression and protection rate. In the present study, two recombinant Marek's disease virus type 1 (MDV1) vaccines containing the VP2 gene of infectious bursal disease virus (IBDV) under control of different promoters were generated from overlapping fosmid DNAs. The rMDV-Pec-VP2 virus containing the VP2 gene under control of the Pec promoter (CMV enhancer and chicken β-actin chimera promoter) demonstrated higher VP2 expression and stronger antibody response against IBDV in chickens than the rMDV-CMV-VP2 virus using the CMV promoter. After IBDV lethal challenge in specific-pathogen-free chickens, rMDV-Pec-VP2 provided complete protection against developing mortality, clinical signs, and the formation of bursal lesions, which was better than that provided by rMDV-CMV-VP2. Our findings indicate that the protective efficacy of the recombinant MDV1 vaccine against IBDV highly correlates with VP2 expression. This recombinant MDV1 vaccine expressing VP2 could have significant potential as a bivalent vaccine against both virulent IBDV and MDV infections in chickens.

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1. Introduction

Infectious bursal disease (IBD) is an acute contagious immunosuppressive disease affecting young chickens. The disease was first described more than 50 years ago and continues to be a threat to the global poultry industry [1]. Vaccination is currently a major strategy for the prevention and control of IBD. However, due to the emergence of variant or highly virulent IBDV strains [2,3], chickens are not fully protected by the current IBD vaccines [4]. Moreover, inactivated and live attenuated vaccines against IBDV carry the intrinsic hazard of being pathogenic due to incomplete inactivation of the virulent virus, or reversal to virulence [5,6]. Maternal antibodies protect chickens up to the age of 3 weeks, but reduce their active immune responses induced by the conventional live vaccines, making the timing of vaccination difficult [7].

Infectious bursal disease virus (IBDV) has a bisegmented dsRNA genome [8,9]. The strictly ordered part of the IBDV capsid is made up exclusively of VP2 [10]. VP2 contains epitopes responsible for eliciting neutralizing antibodies [11]. The problems associated with conventional vaccines may be solved by using subunit vaccines. As the major protective antigen of IBDV, the VP2 gene has been presented by plasmid DNA or produced from various heterologous expression systems [12,13]. However, large immunizing doses and multiple injections are needed to achieve optimal protective efficacy against virulent IBDV challenge, bottlenecking in the development of these vaccines.

Marek's disease (MD) is a neoplastic and neuropathic disease in chickens caused by a highly cell-associated herpesvirus known as Marek's disease virus (MDV) [14]. MDV has a large genome and several regions in its genome are nonessential for viral replication. MDV vaccine can be inoculated into one-day-old field chicks that have high titers of maternal antibodies in order to establish early immunity against vvIBDV infection [15]. Moreover, MDV vaccines can induce lifetime immunity in chickens with just one vaccination. These features therefore make MDV vaccine strain a highly desirable viral vector for expressing the VP2 gene of IBDV for the development of recombinant vaccines.

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In this study, the VP2 gene of vvIBDV was inserted into the genome of MDV1 vaccine strain. To determine the effects of different promoters on the protective efficacy of the recombinant vaccines, two recombinant MDVs using the CMV or CMV/β-actin chimera (Pec) promoter were generated from overlapping fosmids. This study demonstrates that the vaccine efficacy of rMDV1 against IBDV correlates with VP2 expression. The rMDV1 expressing a larger amount of VP2 conferred complete protection against a lethal vvIBDV challenge.

2. Materials and methods

2.1. Viruses and cells

The MDV1 814 vaccine strain [16] was used as the parent virus for producing recombinant MDVs. The vvMDV Md5 and vvIBDV HLJ0504 [17] strains were used as the challenge viruses. MDVs were propagated in chicken embryo fibroblasts (CEFs) prepared from 10-day-old specific-pathogen-free (SPF) chicken embryos.

2.2. Construction of fosmids with VP2 gene insertion

The VP2 gene from vvIBDV HLJ0504 strain was introduced into pCI vector under control of CMV promoter (CMV enhancer/promoter), or pCAGGS vector under control of Pec promoter (CMV enhancer/β-actin promoter). The VP2 cassettes were then used to replace the gus gene in pENTR-gus (Invitrogen) to obtain the attL1 and attL2 arms. Five fosmid clones that contain genomic sequences spanning the entire genome of MDV1 vaccine strain 814 were constructed in our preliminary studies (Fig. 1A). To simplify the insertion of foreign genes into MDV1 genome, fosmid 103 was modified by inserting a dual selection marker encoding kanamycin resistance gene (KanR) and ccdB gene flanked by attR1 and attR2 sequences into US2 gene of MDV1 with the Counter-Selection BAC modification kit (Gene Bridges). To insert VP2 cassette into US2 site, the entry plasmids pENTR-CMV-VP2 and pENTR-Pec-VP2 were mixed with the modified fosmid 103US2-KanccdB and treated with LR Clonase II enzyme (Invitrogen). The mixtures were transformed into competent *Escherichia coli* EPI300-T1 cells. Only fosmids with the KanR-ccdB marker replaced by the VP2 cassette could replicate and be selected in EPI300-T1.

2.3. Rescue of recombinant MDV1 from overlapping fosmid DNAs

Five fosmid combinations with or without VP2 insertions that covered the entire MDV1 genome were used for virus rescue. Viral DNA inserts were released from purified fosmids by digestion with NotI and used to transfect primary CEFs in 60-mm dishes by using the calcium phosphate procedure [18]. The CPE-positive samples were harvested and characterized by electron microscopy. To verify that the VP2 gene was inserted into the MDV1 genome at the desired sites, the viral genomic DNA was analyzed by PCR and sequencing.

2.4. Confirmation and comparison of the VP2 gene expression

VP2 expression from the recombinant MDVs were confirmed by indirect immunofluorescence assay (IFA) and Western blotting using anti-VP2 monoclonal antibody (MAb). To compare VP2 expression levels from different rMDVs, CEF cells were infected with 1000 PFU of the rMDVs and maintained for 120 h before harvesting. After fixed and perforated, the cells were incubated first with mouse anti-VP2 MAb, and then with FITC-conjugated goat anti-mouse IgG antibody. The mean fluorescence intensity (MFI) of VP2 positive cells (10,000 events captured per sample) was assessed by fluorescence-activated cell-sorting (FACS) analysis (FACS Aria; Becton Dickinson).

2.5. Growth properties and stability of the rescued viruses

To investigate the growth property of the recombinant MDVs, cells cultured in 6-well plates were inoculated with 100 PFU of the rescued viruses. The infected cells were harvested at different time points and serial dilutions were inoculated onto fresh CEFs. The plaques of the different dilutions were counted five days later. To evaluate the genetic stability of the recombinant MDVs, viruses

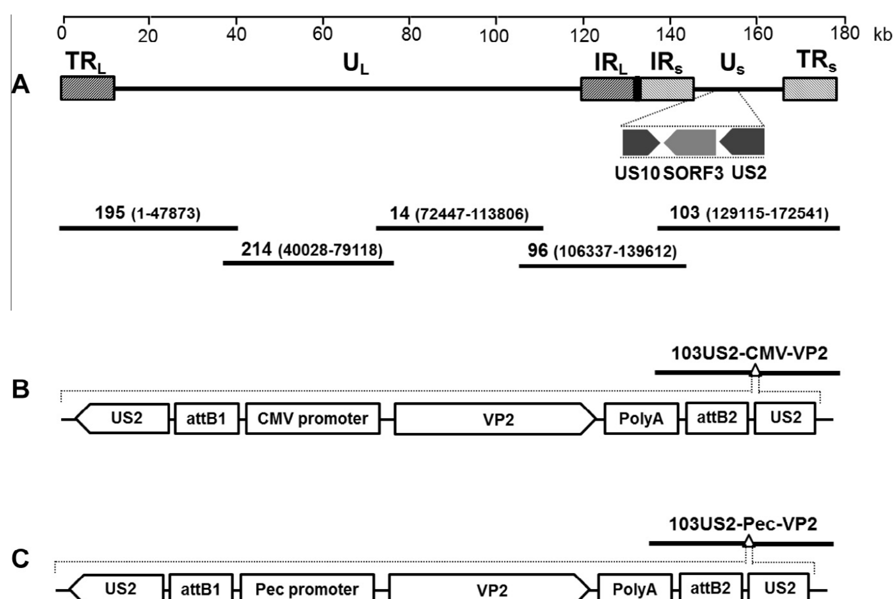

Fig. 1. Construction of fosmids containing VP2 gene under the control of different promoters. (A) The genomic structure of the MDV vaccine strain, and the five fosmid DNAs used for MDV regeneration. Numbers represent the fosmid names and the location of each fosmid fragment in the MDV strain genome. (B) The schematic diagrams of the fosmid containing VP2 gene under control of the CMV promoter. (C) The schematic diagrams of the fosmid containing VP2 gene under control of the Pec promoter (CMV enhancer/β-actin chimera promoter).

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