A RECOMBINANT FOWLPOX VIRUS VACCINE EXPRESSING GLYCOPROTEIN B GENE FROM CVI988/RISPENS STRAIN OF MDV: PROTECTION STUDIES IN DIFFERENT CHICKENS

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Summary. – Recombinant fowlpox virus (rFPV) was constructed to express glycoprotein B (gB) gene from CVI988/Rispens strain of Marek's disease virus (MDV). The rFPV-gB/R alone and in combination with herpesvirus of turkey (HVT) preparations were evaluated for their protective efficacy against challenge with very virulent MDV strains Md5 and RB1B in different chickens. The rFPV-gB/R alone induced protection comparable to that by HVT vaccines in both Ab^- SPF chickens and Ab^+ production chickens. Significant protective synergism was observed in one of these two types of commercial production chickens when rFPV-gB/R was combined with HVT of either cell-associated or cell-free preparations. Immunogenesis studies showed that rFPV-gB/R, just like conventional vaccines, significantly reduced the level of viremia, splenocytes infection and feather follicle shedding of challenge virus in vaccinated chickens.

Key words: recombinant fowlpox virus; vaccine; glycoprotein B; MDV; CVI988/Rispens strain

Introduction

MD is currently controlled by vaccines consisting of three serotypes of MDV, either alone or in bivalent and trivalent combinations (Witter and Lee, 1984). Glycoprotein B (gB) of MDV is the major protective antigen (Ono *et al.*, 1985; Nazerian *et al.*, 1996) and is composed of a precursor of 100 kDa protein and two cleavage products of 60 kDa and 49 kDa glycoproteins (Silva and Lee, 1984). Protection by rFPV vaccines expressing gB gene from MDV in either Ab^- or Ab^+ SPF chickens has been reported (Nazerian *et al.*, 1992, 1996; Heine *et al.*, 1997).

In the previous reports we have cloned and sequenced the gB gene from the well-known vaccine strain CVI988/Rispens of MDV (Xing *et al.*, 1998) and a rFPV expressing the gB gene has been constructed (Liu *et al.*, 1996; Xing *et al.*, 1998). The objective of this study was to evaluate the protective efficacy of rFPV-gB/R either alone or in combination with HVT against challenge with very virulent MDV strains Md5 and RB1B in both commercial SPF chickens and production chickens.

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Materials and Methods

Viruses. Very virulent MDV strains Md5 (Witter, 1980) and RB1B (Schat *et al.*, 1982) were used as challenge viruses. Vaccine viruses included MDV CVI988/Rispens strain (Rispens *et al.*, 1972) of serotype 1, Z4 strain (Huang *et al.*, 1988) of serotype 2 and FC126 strain (Witter *et al.*, 1970) of HVT (serotype 3). Both cell-associated and cell-free stocks of HVT were used. The cell-free HVT vaccine (Nanjing Bioproducts, Nanjing, P.R. China) and CVI988 vaccine (Select Laboratories, Inc., Gainesville, GA, USA) were derived directly from commercial sources and assayed before use.

rFPV-gB/R was constructed as reported elsewhere (Xing *et al.*, 1998). The Chinese vaccine strain 282E4 of FPV used for the construction of rFPVs was included as wild type control. All of these viruses, except where indicated otherwise, were propagated in either chicken embryo fibroblast (CEF) or duck embryo fibroblast (DEF) cultures and titrated.

Chickens. Commercial SPF White Leghorn chickens were derived from the breeder flock of Nanjing Bioproducts and they were maternal Ab^- to MDV, HVT and FPV. Two commercial layer breeds of production chickens, Isabrown and Wolfhill, were known to be susceptible to MD (Liu *et al.*, 1992) and were maternal Ab^- to MDV, HVT and FPV. Hatching eggs were supplied by local breeder farms and incubated in laboratory facilities.

Protection trials. Groups of one-day-old chickens in each trial were vaccinated with 10^6 PFU of rFPV vaccine by intraabdominal

(IA) route. Monovalent and bivalent MDV and HVT vaccines were inoculated by the IA route at a dose of 2000 PFU. For bivalent vaccines composed of either HVT+rFPV or HVT+MDV, the component viruses were mixed and given as a single inoculum. Groups of chickens were housed in isolation after vaccination. Vaccinated and unvaccinated chickens were challenged with a mixture of Md5 and RB1B by IA route at 1000 PFU aggregate dose. Chickens were held up to 84 days post-challenge. Birds dying during the course of experiment and survivors at termination were examined for the presence of MD gross lesions. Calculation of the percentages of MD, protection and synergism was according to Witter *et al.* (1980) and Nazerian *et al.* (1996).

Virus isolation and assay. The level of viremia and splenocyte infection induced by challenge MDVs in chickens were examined by virus isolation or rescue. The methods for preparation of peripheral blood lymphocytes (PBLs) and splenocytes suspensions have been described elsewhere (Witter and Lee, 1984; Schat *et al.*, 1982). PBLs or splenocytes were seeded to secondary DEF or CEF monolayers on 24-well tissue culture plates at a dose of 2 x 10^5 cells per well, with three replicate wells for each sample, and allowed to incubate for two days. Medium was changed at this time. After additional 4 days of incubation, the culture plates were subjected to immunofluorescence staining (Uchanska-Ziegler, 1980) using MDV serotype 1 specifife monoclonal antibody (Lee *et al.*, 1983). Plaques produced by the challenge MDVs in cultures were counted under immunofluorescence microscope.

Statistical analysis. Quantitative data of means were analyzed by Student's *t*-test and mortality and protection data were compared by chi square test.

Results and Discussion

Protective immunity against MD

Results of three trials are summarized in Tables 1-3. All vaccines including monovalent rFPV-gB/R and two monovalent HVT preparations provided high level of protection in Ab$^-$ SPF chickens in the range of 52.7–71.4% (Table 1), however, the magnitude of the protection between the vaccines did not differ significantly. In two commercial breeds of production chickens, the rFPV-gB/R vaccine alone induced 20.7–28.0% protection, similar to that induced by cell-free HVT (19.1–26.1%) and cell-associated HVT (21.3–40.0%), although the cell-associated HVT vaccine was obviously better in trial 3 (Tables 2 and 3). The protective efficacy of the bivalent vaccines composed of rFPV-gB/R and cell-associated or cell-free HVT was significantly higher than those of the single component vaccines (17.1–21.5% enhancement) and approached the protection level afforded by conventional serotype 2 and 3 bivalent vaccine and serotype 1 CVI988 vaccine in Isabrown chickens (Table 2). The enhancement of protection between rFPV-gB/R and HVT was less obvious in Wolfhill chickens (Table 3), probably because the component vaccines induced higher protection than in trial 2. Pro-

Table 1. Protection of Ab$^-$ SPF chickens against challenge with mixture of Md5 and RB1B strains of MDV after vaccination with rFPV-gB/R or conventional vaccines (trial 1)

Vaccine	Replicate 1		Replicate 2		Summary	
	MD$^+$/total	PI	MD$^+$/total	PI	MD$^+$/total	PI[a]
rFPV-gB/R	10/19	47.4	8/20	57.9	18/39	52.7a
rFPV-gB/R+HVT (CA)	8/20	57.9	7/19	61.3	15/39	60.6a
rFPV-gB/R+HVT (CF)	9/19	52.5	5/22	76.1	14/41	65.1a
HVT (CA)	10/22	54.5	8/22	61.7	18/44	58.1a
HVT (CF)	8/21	61.9	7/19	50.1	17/40	56.5a
HVT+Z4	11/21	47.6	7/20	63.2	18/41	55.0a
CVI988	7/22	68.6	5/21	74.9	12/43	71.4a
FPV (wt)	15/15	0.0	13/15	8.7	28/30	3.5b
None	21/19	–	19/20	–	40/41	–

CA = cell-associated; CF = cell-free.
PI = protective index.
[a]Values followed by the same letter do not differ significantly (P <0.05).

Table 2. Protection of Isabrown breed of commercial production chickens against challenge with mixture of Md5 and RB1B strains of MDV after vaccination with rFPV-gB/R or conventional vaccines (trial 2)

Vaccine	Replicate 1		Replicate 2		Summary		
	MD$^+$/total	PI	MD$^+$/total	PI	MD$^+$/total	PI[a]	Synergism (%)
rFPV-gB/R	16/22	20.7	22/35	25.8	40/57	22.8a	
rFPV-gB/R+HVT (CA)	9/24	50.0	13/35	56.3	21/59	57.66b	121.5
rFPV-gB/R+HVT (CF)	3/27	47.5	14/34	51.4	27/61	49.5b	117.1
HVT (CA)	15/24	31.7	22/33	21.3	37/57	26.0a	
HVT (CF)	16/23	24.0	24/35	19.1	40/58	21.3a	
HVT+Z4	11/22	45.4	10/35	66.3	21/57	58.0b	
CVI988	10/26	58.0	13/32	52.1	23/58	54.7b	
None	22/24	–	28/33	–	50/57	–	

CA = cell-associated; CF = cell-free; PI = protective index.
[a]Values followed by the same letter do not differ significantly (P >0.05).

Table 3. Protection of commercial production chickens (Wolfhill) against challenge with mixture of Md5 and RB1B strains of MDV with rFPV-gB or conventional vaccines (trial 3)

Vaccine	Replicate 1		Replicate 2		Summary		
	MD^+/total	PI	MD^+/total	PI	MD^+/total	PI[a]	Synergism (%)
rFPV-gB/R	17/24	25.9	18/25	28.0	35/49	27.0bc	
rFPV-gB/R+HVT (CA)	9/25	62.3	10/24	58.3	19/49	60.3d	54.0
rFPV-gB/R+HVT (CF)	12/24	47.6	13/23	43.5	25/47	45.6cd	68.9
HVT (CA)	13/22	38.1	15/25	40.0	28/47	39.1bcd	
HVT (CF)	19/25	20.4	17/23	26.1	36/48	23.3abc	
HVT+Z4	11/24	52.0	9/24	62.5	20/48	57.4d	
CVI988	12/23	45.3	8/24	66.7	20/47	8.4a	
None	21/22	–	23/23	–	44/45	–	

CA = cell-associated; CF = cell-free; PI = protective index.
[a]Values followed by the same letter do not differ significantly ($P < 0.05$).

Table 4. Effect of vaccines on the level of MDV viremia induced by challenge viruses

Vaccine	MDV viremia at given days post challenge[a]							
	7		21		35		56	
	V^+/T[b]	PFU[c]	V^+/T	PFU[c]	V^+/T	PFU[c]	V^+/T	PFU[c]
rFPV-gB/R	1/5	0.5a	4/5	12.4bc	3/5	8.7bc	3/5	3.2b
rFPV-gB/R+HVT (CA)	0/5	0.0a	2/5	4.3a	1/5	1.9a	1/5	0.9a
rFPV-gB/R+HVT (CF)	1/5	0.2a	3/5	4.5a	2/5	4.7ab	1/5	0.3a
HVT (CA)	2/5	0.8a	5/5	8.3a	2/5	2.9a	1/5	1.1ab
HVT (CF)	0/5	0.0a	5/5	13.8bc	3/5	6.7ab	2/5	1.3ab
HVT+Z4	1/5	0.4a	3/5	9.6b	4/5	7.4bc	3/5	2.1ab
CVI988	1/5	0.2a	4/5	7.2b	3/5	6.5ab	2/5	0.7a
FPV (wt)	5/5	6.3b	5/5	50.0d	5/5	35.3d	2/5	2.2ab
None	5/5	8.4b	5/5	46.1d	5/5	65.4d	1/5	0.3a

[a]Birds in this test were from protection trial 1.
[b]V^+ = number of chickens with viremia; T = total number of chickens tested.
[c]Mean number of plaquesrecovered; values followed by the same letter do not differ significantly ($P > 0.05$).

Table 5. Effect of vaccines on the rescue of challenge MDV from splenocytes of chickens

Vaccine	Rescue of MDV at given days post challenge[a]		
	5	19	33
	Mean number of PFU/2 x 10^5 splenocytes[b]		
rFPV-gB/R	0.7a	2.2a	1.8a
rFPV-gB/R+HVT (CA)	0.1a	1.2a	0.2a
rFPV-gB/R+HVT (CF)	0.3a	1.3a	1.5a
HVT (CA)	1.1a	1.4a	2.6ab
HVT (CF)	0.8a	2.0a	3.0ab
HVT+Z4	0.0a	1.2a	2.6ab
CVI988	3.9a	0.4a	0.4a
FPV (wt)	20.0b	27.0b	25.0c
None	17.7b	30.0b	29.0c

[a]Extra chickens in each group of protection trial 2 were allocated and used in this test.
[b]Values in the same column followed by the same letter do not differ significantly ($p > 0.05$).

tection and synergism by rFPV vaccines expressing genes from MDV have been reported (Nazerian *et al.*, 1996). rFPV/gB1 protected Ab^+ $15I_5X7_1$ SPF chickens with efficacy similar to that of cell-associated HVT and that protection was enhanced significantly when rFPV/gB1 was combined with HVT. More recently Heine *et al.* (1997) reported that rFPV-gB vaccine failed to protect commercial production chickens against MD, although it did protect Ab^- SPF chickens. The results of the comparative efficacy study presented here clearly indicate that the rFPV-gB/R vaccine provided substantial protection against MD in both Ab^- commercial SPF chickens and Ab^+ commercial production chickens and protective synergism between rFPV-gB/R and HVT existed in chickens of certain genetic background under certain test conditions.

Immunogenesis of rFPV-gB/R vaccine

The results showed that rFPV-gB/R, similar to conventional vaccines, caused significant and sustained reduction in the level of viremia, splenocyte infection and feather follicle shedding by challenge viruses in vaccinated chickens when compared with the unvaccinated but challenged birds (Tables 4-6). The level of viremia from 7 to 35 days post-challenge in vaccinated chickens was significantly lower than that in unvaccinated chickens. The rescue of challenged MDV from splenocytes at 5, 19 and 33 days post-challenge showed that vaccination greatly suppressed the infection and replication of MDV in spleen cells. From 11 days to 35 days post-challenge, all unvaccinated chickens sheded MDV heavily, how-

Table 6. Effect of vaccines on the level of challenge virus shedding from feather follicles of chickens detected by agar gel precipitation (AGP) test

Vaccine	Days post challenge[a]									
	11		14		21		28		35	
	SS	S^+/T	SS	S^+/T	SS	S^+/T	SS	S^+/T	SS	S^+/T
rFPV-gB/R	0.00	0/9	0.38	3/9	0.00	0/9	0.33	3/9	0.67	5/9
rFPV-gB/R+HVT (CA)	0.00	0/9	0.00	0/9	0.00	0/9	0.58	6/9	0.33	4/9
rFPV-gB/R+HVT (CF)	0.00	0/9	0.33	4/9	0.08	1/9	0.50	5/9	0.61	7/9
HVT (CA)	0.00	0/9	0.08	1/9	0.00	0/9	0.08	1/9	0.00	0/9
HVT (CF)	0.00	0/9	0.66	6/9	0.00	0/9	0.25	3/9	0.00	0/9
HVT+Z4	0.08	1/9	0.00	0/9	0.00	0/9	0.00	0/9	0.33	2/9
CVI988	0.00	0/9	0.16	2/9	0.00	0/9	0.00	0/9	0.08	1/9
FPV (wt)	0.08	1/9	0.70	7/9	1.00	9/9	0.83	8/9	1.00	9/9
None	0.75	9/9	0.91	9/9	1.00	9/9	1.00	8/9	1.00	/9

[a]Chickens for this test were those in protection trial 1. For AGP test, 1% agar plates were used and six feather tips from each bird were collected to detect MDV antigens.

SS = shedding score, the sum of positive tips divided by the sum of tips tested in a group.

S^+/T = number of chickens that were positive over total number of chickens tested in a group. To be regarded as positive bird, only one positive tip was needed.

ever, the shedding of MDV in vaccinated birds was postponed and was less severe. It seems that the rFPV-gB/R, just like conventional MD vaccines, protected chickens against early replication of virulent MDV in the lymphoid organs and reduced the level of latent infection.

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