Evaluation of Pathogenicity and Protective Efficacy of Serotype 2 Marek's Disease Virus from Birds Belonging to Genus *Gallus* in Japan

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ABSTRACT. The new cloned serotype 2 Marek's disease viruses (MDV) of ML-6, ML-9, and ML-22 strains were inoculated in specific-pathogen-free (SPF) chicks to evaluate the pathogenicity and protective efficacy. Chicks inoculated or contact-infected with ML strains showed no gross and histological lesions in lymphoid organs, sciatic plexuses and other visceral organs during 10 weeks of observation periods, indicating that the viruses were non-pathogenic. Moreover, the viruses were found to be spread horizontally among chicks by demonstrating the presence of viremia in contacted chicks at 2 weeks-old. Chicks vaccinated with ML-6 at one day-old were protected against subsequent challenge by inoculation with virulent MDV strain of Md/5 at 4 or 7 days old or by contact infection at 7 days old with chickens previously inoculated with the same strain.—**KEY WORDS:** pathogenicity, protection, serotype 2 MDV.

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Marek's disease (MD), a naturally occurring lymphomatous neoplasm of fowls, was first reported in 1907 by Josef Marek [8]. Virtually all commercial chickens develop the disease, and it had been considered to be the most serious disease which afflicted poultry farms. A large number of MDV strains had been isolated, and these strains were divided into different serotypes on the basis of serological analysis with indirect immunofluorescence (IF), agar gel precipitation (AGP) and virus neutralization (VN) tests [1–3]. Pathogenic or oncogenic strains of MDV and attenuated viruses of these strains belonged to serotype 1 whereas apathogenic or non-oncogenic strains belonged to serotype 2. Serologically related herpesvirus isolated from turkey (HVT) was classified as serotype 3.

In recent years, laboratories all over the world have contributed to finding of the virus strains for immunization against virulent Marek's disease viruses (MDVs). Up to date, FC-126 strain of HVT [13], SB-1 strain

of serotype 2 MDV [12], CVI 988 strain of serotype 1 MDV [11], and others are currently available in the vaccine market. Since then, vaccination of commercial chickens was routinely practiced at 1 day of age, and this program has markedly reduced the incidence of the disease. Recently, increased incidence of MD in vaccinated flocks was observed in many parts of the world. The various factors associated with these problems were considered. Vaccination control programs including choice of vaccine, type of administration, and ancillary factors were discussed to control vaccine breaks of MD.

A previous report [7] had discussed some of the preliminary findings. Thirteen strains of serotype 2 MDV were isolated from some of the apparently healthy birds including a golden pheasant, white silky fowls, black silky fowls, Japanese long crows and Japanese bantams. Three cloned isolates, designated as ML-6 (black silky fowl), ML-9 (Japanese long crower), and ML-22 (golden

pheasant), were characterized following isolation. In order to know the *in vivo* characteristics of ML strains, this paper describes the experiments of pathogenic effect and protective ability of ML strains against subsequent infection of virulent Md/5 in experimental chicks. Also, SB-1 was used to vaccinate chicks to compare with the protective ability of ML strains through contact infection with Md/5.

MATERIALS AND METHODS

Cell culture system: Chicken embryo fibroblast (CEF) and chicken kidney cell (CKC) cultures were prepared from chicken embryonated eggs and kidneys from specific-pathogen-free (SPF) chickens (line LMG), respectively, as described previously [5].

Virus: Cloned ML-6, ML-9, and ML-22 strains of serotype 2 MDVs isolated from a black silky fowl, Japanese long crower and golden pheasant, respectively [7], were harvested from CEF cultures and used for evaluation of pathogenic effect and protective efficacy. The Md/5 strain of serotype 1 MDV [14] and SB-1 strain of serotype 2 MDV [12] were harvested from CKC and CEF cultures, respectively, and used as reference viruses in this experiment.

Experimental chickens: The SPF chicks (line LMG), supplied by Aburahi Laboratories, Shionogi & Co., Ltd., Shiga, were used as source of experimental chickens in this experiment.

Pathology: Records were kept for all chickens dying during the experiments. In critical laboratory experiments, all dead chickens were necropsied. If no gross diagnosis could be made, sciatic plexuses, spleen, thymuses, bursa of Fabricius, heart, liver, proventriculus and brain were examined microscopically, using paraffin section and hematoxylin-eosin (HE) staining techniques.

Virus isolation: Spleen cells or peripheral blood lymphocytes (PBL) from experimental chickens were inoculated into CEF cultures for virus isolation. Spleen was gently forced through a 150 (#) stainless steel mesh and washed 2 times with Eagle's minimum essential medium (Nissui, Japan) with the addition of 1% fetal calf serum and 10% tryptose phosphate broth (Difco, USA). The spleen cells were separated by centrifugation over Ficoll-Conray [5, 6]. Blood lymphocytes were also isolated from heparinized blood over Ficoll-Conray. The results of isolation were expressed as plaque-forming-units (PFU) per 10^6 viable cells.

Serological procedures: The IF test was done in the same manner as described by Onuma *et al.* [9].

Experimental design:

Experiment 1 (pathogenicity experiment)

Day-old chicks were divided into 6 groups and treated as follows. Groups 1, 2 and 3 were inoculated intraperitoneally (IP) with 600 PFU of ML-6, ML-9, and ML-22 strains, respectively, and kept in a big isolation pen. Group 4 served as contact group was placed in the same isolation pen as above. Group 5 served as positive control was infected with 600 PFU of Md/5 strain *via* IP and kept in Isolator A. Group 6 served as uninfected control was kept in Isolator B. All birds were observed for 10 weeks. At 2 or 4 weeks of age, 2 birds from each group were necropsied for examining MD lesions. All remaining live chickens were necropsied at the end of 10 weeks old. Virus isolation was made from the spleens or PBL at 2 weeks old by the method described previously [5].

Experiment 2 (protection experiment)

Day-old chicks were divided into 7 groups. Groups 1, 2 and 5 were vaccinated *via* IP with 600 PFU of ML-6 strain after hatching and kept in a big isolation pen. At 4th and 7th days post-vaccination, groups 1

and 2 were challenged subcutaneously (SC) with 800 PFU of Md/5 strain and then moved to Isolator A. Unvaccinated groups 3 and 4 in Isolator B were also challenged with 800 PFU of Md/5 strain *via* SC at 4 and 7 days-old and then moved to Isolator A. Group 5 served as vaccinated-unchallenged group stayed in the original isolation pen. Group 6 served as positive control group was infected *via* IP with 800 PFU of Md/5 strain at one day old and kept in Isolator A. Group 7 served as unvaccinated-unchallenged control was kept in Isolator B. At 4 weeks old, 2 or 3 chicks from each group were necropsied for examining MD lesions. All of the live chickens were necropsied at the end of 6 weeks old.

Experiment 3 (comparative efficacy)

Day-old chicks were divided into 4 groups. Groups 1 and 2 were vaccinated *via* IP with 800 PFU of ML-6 and SB-1 strains, respectively, after hatching and kept in a big isolation pen. Groups 3 and 4 were not vaccinated and kept in the same isolator. At 7 days old, groups 1 and 2 were challenged through contact infection using the Md/5 strain-inoculated chicks of 3 weeks old with MD lesions of feather follicles pre-examined. Group 4 served as positive control group was moved to the same isolation pen as groups 1 and 2. Group 3 stayed in the isolator served as negative control. At 5 and

7 weeks old, chicks were necropsied for examining MD lesions.

RESULTS

Pathogenicity experiment: As shown in Table 1, all birds of groups 1, 2 and 3 inoculated with ML strains did not show mortality or any gross lesions of MD in sciatic plexuses and visceral organs until 10 weeks of age. The birds of group 4 in the same isolation pen as ML strains-inoculating group were kept as contact control group, but they showed no gross lesions of MD or mortality. However, all of the birds of group 5 inoculated with virulent virus of Md/5 strains had evident gross lesions and 100% mortality until the end of experiment.

By IF test, sera of each group showed positive reaction to the serotypes of inoculated MD virus. The bursa of Fabricius was sectioned for histological examination. In either 2 or 4 weeks after inoculation, the birds of groups 1, 2, 3 and 4 were entirely free of follicle damage (Table 2). The ratios of bursa to body weight were almost the same as normal control group 6. On the other hand, the birds of group 5 showed 26% and 28% of follicle damage in 2 and 4 weeks after inoculation, respectively. Also, the ratio of bursa to body weight was very low compared with normal control group 6.

Table 1. Incidence of MD in chickens exposed to ML-6, ML-9, ML-22 and Md/5 strains in 10 weeks observation (experiment 1-1)

Group	Housing	No. of birds	Inoculation at 1 day old				No. of birds with gross lesion	%MD Mort. in 10 wks observation	Serology (IF test)
			Status ^{a)}	Virus	Dose ^{b)}	Route ^{c)}			
1	Iso. pen	10	Inf	ML-6	600	IP	0	0	10/10
2	"	10	Inf	ML-9	600	IP	0	0	10/10
3	"	10	Inf	ML-22	600	IP	0	0	10/10
4	"	10	CC	—	—	—	0	0	10/10
5	Isolator A	10	Inf	Md/5	600	IP	10 ^{d)}	100	10/10
6	Isolator B	10	NC	—	—	—	0	0	0

a) Inf: infected, CC: contact control, NC: negative control.

b) Dose: PFU per bird.

c) IP: Intraperitoneally.

d) Gross lesion of visceral lymphomas in ovary, heart, kidney or proventriculus.

Table 2. Degenerative lesions in the bursa of Fabricius exposed to ML-6, ML-9, ML-22 and Md/5 strains inoculated at one day old (experiment 1-2)

Group	Virus	No. of birds	% Follicle damage ^{a)}		Bursa wt/total body wt ^{b)}		
			2wks	4wks	2wks	4wks	10wks
1	ML-6	8	0	0	0.011	0.012	0.005
2	ML-9	8	0	0	0.011	0.013	0.005
3	ML-22	8	0	0	0.010	0.013	0.005
4	—	8	0	0	0.010	0.012	0.005
5	Md/5	4	26	28	0.003	0.007	— ^{c)}
6	—	8	0	0	0.012	0.013	0.006

a) Percent of bursal follicles with severe depletion of lymphocytes, necrosis, or atrophy; at least 50 follicles per bird were evaluated; mean of 2 birds at 2 and 4 weeks old.

b) Mean of 2 birds sampled at 2 and 4 weeks old, and 4 birds at 10 weeks old if lived.

c) No birds lived.

These results indicated that the ML strains were non-pathogenic and non-oncogenic for line LMG chickens during 10 weeks of observation periods, when inoculated at one day old.

As shown in Table 3, the viruses were recovered from spleen cells or PBL from chickens infected with ML strains at 2 weeks after inoculation. Moreover, the birds (of group 4) reared together with the ML strain-inoculated birds were also shown to be horizontally infected through contact infection.

Protection experiment: The results of protection experiment are summarized in Table 4. Although, the challenge was made in either 4 or 7 days after vaccination, the vaccinated chicks of groups 1 and 2 showed no mortality until 6 weeks of age. The body and bursa weights of these 2 groups were similar to those of non-challenged chickens of group 5. After challenging, the non-vaccinated chickens of groups 3 and 4 presented more poor growing appearance and lighter bursa weight in comparison with all vaccinated groups at 6 weeks of age. Although no mortality was noted until the end of experiment, the MD lesions of non-vaccinated chickens were more obvious by gross or histological examination.

Table 3. Virus recovery from spleen cells and peripheral blood lymphocytes from chickens infected with ML-6, ML-9, ML-22 and Md/5 strains at 2 weeks after inoculation (experiment 1-3)

Group	Virus	PFU/10 ⁶ cells ^{a)}	
		Spleen cells	Blood lymphocytes
1	ML-6	254	74
2	ML-9	40	14
3	ML-22	35	4
4	—	25	12
5	Md/5	42	20
6	—	0	0

a) Either spleen cells or blood lymphocytes were pooled from 2 birds, and inoculated into CEF. Plaques were counted 5 days for ML strains, and 7 days for Md/5 strain after inoculation. The plaques were expressed as PFU per 10⁶ cells inoculated.

Table 5 shows the MD incidence of gross and histological lesions in nerves of sciatic plexuses, lymphoid organs of bursa, thymus and spleen, or other visceral organs. Only mild degenerative changes of bursa and inflammatory lymphocytic infiltration of nerves were seen in about half of the chicks of group 1 or 2. On the other hand, all of the non-vaccinated chicks of groups 3 and 4 showed severe lymphoid depletion of lymphoid organs, obvious neoplastic lymphocytic proliferation of sciatic nerves and visceral lymphoma. Non-challenged chicks of

Table 4. Protection in ML-6 strain-vaccinated chickens against direct challenge with Md/5 strain during 6-weeks observation (experiment 2-1)

Housing ^{a)}					Inoculation at day-old		Challenge ^{c)}		No. of positive ^{d)} birds/No. of birds necropsied			Appearance			Specific mortality in 6 wks observation
Before challenge		After challenge		Status	Dose ^{b)}	Status	Days post-vaccination	birds/No. of birds necropsied			Body wt(gm)		Bursa wt at 6wks		
Group								4wks	6wks	Total	4wks	6wks		6wks	
1	Iso. pen	Iso. A	Vac	600	+	4	2/3	1/2	3/5	217	330	1.32	0/5		
2	Iso. pen	Iso. A	Vac	600	+	7	1/3	1/2	2/5	190	345	1.43	0/5		
3	Iso. B	Iso. A	—	—	+	4	3/3	1/1	4/4	203	270	0.88	0/4		
4	Iso. B	Iso. A	—	—	+	7	3/3	2/2	5/5	182	260	0.71	0/5		
5	Iso. pen	Iso. pen	Vac	600	—	—	0/3	0/3	0/6	172	335	1.49	0/6		
6	Iso. A	Iso. A	Md/5	800	—	—	4/4	ND ^{e)}	4/4	144	162	0.65	1/5 ^{f)}		
7	Iso. B	Iso. B	—	—	—	—	0/3	0/2	0/5	200	390	2.00	0/5		

a) Iso. pen: isolation pen, Iso.: isolator.

b) Dose: PFU per bird.

c) +: challenged with 800 PFU per bird, —: not challenged.

d) Birds showed gross or histopathological lesions in 4 or 6 weeks observation.

e) ND: not done.

f) One bird died at 25 days old with lesions.

Table 5. Incidence of MD in ML-6 strain-vaccinated chickens against direct challenge with Md/5 strain in 6 weeks observation (experiment 2-2)

Group	Age(wk) when examined	No. of birds	No. of birds with MD lesions								
			Gross lesions ^{a)}			Microscopic lesions					
			Deg	NI	VI	Necrosis or atrophy		Lymphoid hyperplasia		Nerve ^{b)}	
						Bursa	Thymus	Spleen	Other viscera	A-type	C-type
1	4	3	1	0	0	2	0	0	0	0	0
	6	2	0	0	0	0	0	0	0	0	1
2	4	3	0	0	0	1	0	0	0	0	0
	6	2	0	0	0	1	0	0	0	0	0
3	4	3	2	1	1	3	3	3	3	2	1
	6	1	1	1	1	1	1	1	1	0	1
4	4	3	3	2	3	3	3	3	3	3	0
	6	2	2	1	2	2	2	2	2	0	2
5	4	3	0	0	0	0	0	0	0	0	0
	6	3	0	0	0	0	0	0	0	0	0
6	4	4	4	3	4	4	4	4	4	3	1
	6	ND ^{c)}	ND	ND	ND	ND	ND	ND	ND	ND	ND
7	4	3	0	0	0	0	0	0	0	0	0
	6	2	0	0	0	0	0	0	0	0	0

a) Deg: degenerative lesions of the bursa of Fabricius and thymus; NI: gross enlargement of sciatic plexuses; VI: leukotic tumors in the visceral organs.

b) A-type lesions considered to be proliferative (neoplastic); C-type lesions interpreted as inflammatory.

c) ND: not done.

Table 6. Comparative efficacy of protection in ML-6 and SB-1 strain-vaccinated chickens against contact infection with Md/5 strain-inoculated chickens at 7 days old during 7 weeks observation (experiment 3-1)

Group	No. of bird	Housing		Vaccination at 1 day old		No. of positive ^{c)} birds/No of birds necropsied			Growth condition ^{d)}						Specific mortality in 7 wks observation
		Before cont inf ^{a)}	After cont inf	Status ^{b)}	Dose	5wks	7wks	Total	5wks			7wks			
									No of birds	Body wt	Bursa wt	No of birds	Body wt	Bursa wt	
1	22	Inf pen ^{a)}	Inf pen	ML-6	800	3/18	1/4	4/22	18	208.06±1.23	1.22±0.14 ^{e)}	4	391.25±5.85 ^{e)}	2.13±0.56 ^{e)}	0/22
2	27	Inf pen	Inf pen	SB-1	800	10/23	1/4	11/27	23	187.61±1.24	0.88±0.10	4	297.50±4.90	1.44±0.48	0/27
3	12	Isolator	Isolator	NC	—	0/10	0/2	0/12	10	216.50±1.72 ^{e)}	1.57±0.16 ^{e)}	2	337.50±4.20 ^{e)}	2.00±0.42 ^{e)}	0/12
4	11	Isolator	Inf pen	PC	—	5/6	2/2	7/8	6	184.16±2.10	0.87±0.16	2	247.50±1.89	1.49±0.25	3/11 ^{f)}

a) cont inf: contact infection, Inf pen: infection pen.

b) NC: negative control, PC: positive control.

c) Birds showed gross or histopathological lesions in 5 or 7 weeks observation.

d) Mean weights of necropsied birds±SD(gm).

e) Significantly different from group 4 at p<0.05.

f) 3 birds died with gross and histopathological MD lesions.

Table 7. Incidence of MD in ML-6 and SB-1 strain-vaccinated chickens against contact infection with Md/5 strain-inoculated chickens at 7 days-old in 7 weeks observation (experiment 3-2)

Group	Age(wk) when examined	No. of birds	No. of birds with MD lesions										Ff ^{c)}
			Microscopic lesions										
			Gross lesions ^{a)}			Necrosis or atrophy		Lymphoid hyperplasia		Nerve ^{b)}			
			Deg	NI	VI	Bursa	Thymus	Spleen	Other viscera	A-type	C-type		
1	5	18	1	0	0	2	3	1	0	0	1	2	
	7	4	0	0	0	0	0	1	0	0	0	1	
2	5	23	4	1	3	5	3	3	2	1	6	10	
	7	4	0	0	0	0	0	0	0	0	1	1	
3	5	10	0	0	0	0	0	0	0	0	0	0	
	7	2	0	0	0	0	0	0	0	0	0	0	
4	5	6	4	2	1	5	4	4	1	3	2	5	
	7	2	2	1	0	2	2	2	0	1	1	2	

a) Deg: degenerative lesions of the bursa of Fabricius and thymus; NI: gross enlargement of sciatic plexuses; VI: leukotic tumors in the visceral organs.

b) A-type lesions considered to be proliferative (neoplastic); C-type lesions interpreted as inflammatory.

c) Ff: Lymphoid cells accumulating and surrounding feather follicles.

groups 5 and 7 were entirely free from MD lesions.

In group 6 as positive control, all of chicks showed MD lesions by gross or histological examination (Tables 4 and 5), but early death at 25 days old with specific lesions was found only one in five chicks.

Comparative efficacy: As shown in Table 6, after challenging through contact infec-

tion at 7 days old, most of chickens were necropsied to examine growth conditions and MD lesions at 5 weeks of age. All remaining live chickens were necropsied at the end of 7 weeks old. The ML-6 vaccinated chicks of group 1 showed normal body and bursa weights which were significantly different from Md/5 inoculated chicks of group 4 ($p<0.05$) which showed poor

growth, relatively high mortality and severe MD lesions in visceral organs or feather follicles examined. The SB-1 vaccinated chicks of group 2 presented poor body and bursa weights as Md/5 inoculated chicks of group 4 did. It was noted in Table 7 that by gross or histological examination, ML-6 vaccinated group presented only mild degenerative changes in bursa and thymuses, and lymphoid hyperplasia of spleen, or inflammatory lymphocytic infiltration of nerves. Obvious MD lesions were found in lymphoid organs or nerves of SB-1 vaccinated group. The accumulation of lymphoid cell around feather follicles or in dermis was more severe than ML-6 vaccinated group.

DISCUSSION

ML strains were isolated from birds belonging to genus *Gallus* (Gallus-birds) and characterized as MD viruses of serotype 2 in previous report [7]. Experiments reported here give evidence that ML strains do not induce inflammatory or neoplastic lesions in inoculated-SPF chickens. As seen from experiment 1, the non-inoculated chicks contacted with ML strains-inoculated chicks did not reveal any gross or histological MD lesions, but the viruses were isolated from contact infected-group (Table 3). These results show that viruses of ML strains are non-pathogenic and can spread horizontally among chickens.

Cho and Kenzy [4] isolated 10 isolates of MDV from 10 zoo birds including Japanese black- or red-face silkies, red jungle fowls, and Ceylon jungle fowls. Six of the 10 isolates were tested for their pathogenicity in a highly susceptible line of chickens, and all failed to cause clinical MD, gross MD lesions or MD deaths. The isolates spread readily from inoculated birds to contact cagemates. In this experiment, 3 isolates (ML strains, identified as serotype 2 by IF test [7]) from Gallus-birds used in

pathogenicity test showed no mortality or MD lesions in gross (Table 1) or microscopic examination (not shown in data) on chicks. The ML strains spread to chicks by contact (Table 3). These results suggest that isolates of MDV from zoo or Gallus-birds are almost non-pathogenic to chickens, and could be spread to cagemates horizontally.

Of 23 birds examined in the previous report [7], over 50% was naturally infected with serotype 2 MDV. These results also indicated that the birds infected by serotype 2 MDV primarily belonged to genus *Gallus* of the order of *Galliformes*. In this experiment, the chickens belonging to genus *Gallus* were readily infected by ML strains. The results show that the genus *Gallus* of either birds or chickens seems to be naturally susceptible to serotype 2 MDV.

Furthermore, ML-6 strain can give relatively good protection against subsequent infection of a virulent MDV, when the chicks were vaccinated at one day old. However, as shown in Table 5, mild lesions were noted in bursa and sciatic plexus which made the differences of body and bursa weights between group 1 & 2 of ML-6 strain-vaccinated chickens and group 7 of negative control. The same results were also found in SB-1 strain-vaccinated chickens (Table 7). We consider that the serotype 2 MDV showed partial protective ability against subsequent infection of a virulent MDV. The partial protective ability of SB-1 strain was also described by Schat *et al.* [12]. Although no lesions were observed in group 5 of vaccinated-unchallenged chickens, the birds showed lighter body and bursa weights than group 7. The inoculation of ML-6 strain is considered to be the possible factor that affects the appearance of growth, while vaccinated at one day old. Also, other unknown factors are suggested to be studied by advanced experiments.

Neural lesions associated with mild inflammatory but not neoplastic changes were

regarded as C-type lesion [10]. SB-1 [12] and high doses of HVT [13] caused C-type lesions which were considered to provoke the immune system of the chicken to mount a vigorous response. The possible explanation would be that non-oncogenic MDV and HVT to have a low potential for transformation which provides the code for MATSA [12]. In our experiments, only few mild C-type lesions were found in the sciatic plexus of ML-6 strain-inoculated chickens (Tables 5 and 7). The mechanism of immune response in non-pathogenic or non-oncogenic MDV of ML-6 and SB-1 strain might be considered by the presence of C-type lesions. Further study is needed to look for the presence of MATSA in the tissues of ML-6 strain-inoculated chickens to explain the hypothesis of immune response.

SB-1 used in this experiment was passaged in CEF more than 6 times since it was obtained from other laboratory. Comparing with the ML-6 strain, the protection efficacy was not as good as expected. On the other hand, the ML strain could provide relatively good protection against subsequent challenge of Md/5 strain (Tables 6 and 7).

In protection experiment, ML-6 of ML strains was used as an example in this study. Because ML strains shared cross antigenic relationship in VN and AGP tests and the similar restriction endonuclease cleavage patterns of viral DNAs [7], all ML strains were considered to be the related viruses. Therefore, ML-9 and ML-22 might possess the same protective efficacy as ML-6 on chicks.

In conclusion, the *in vivo* experiments indicate that ML strains are non-pathogenic to chickens and have protection ability against virulent MDV. We also observed that the birds described in previous report [7] never showed MD death. The serotype 2 MDV existed in the population caused earlier natural infection in the birds, and

therefore they were protected against subsequent infection with virulent MDV.

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観賞用鳥類から分離された血清型 2 マレック病ウイルスの病原性及び免疫予防効果：林 子恩・児玉 洋・板倉智敏¹⁾・小沼 操・見上 彪²⁾(北海道大学獣医学部家畜伝染病学講座, ¹⁾比較病理学講座, ²⁾東京大学農学部獣医微生物学講座)——血清型 2 マレック病ウイルス ML 株 (ML-6, ML-9, 及び ML-22) の SPF 実験鶏に対する病原性及び発病防御効果を検討した。10 週齢までの観察期間内, ML 株接種あるいは接触感染した鶏のいずれにもリンパ性器官, 坐骨神経叢及び内部臓器に肉眼及び組織病変は見られず, これらウイルス株は非病原性であった。2 週齢の接触感染鶏でウイルス血症が観察され, これら ML 株は水平感染を起こした。ML-6 を 1 日齢ヒナに免疫すると, 4 あるいは 7 日齢に強毒 Md/5 株接種による攻撃に対し, および 7 日齢に Md/5 株感染鶏による接触感染攻撃に対し, 明らかな発病防御効果を示した。