# Detection of Marek's Disease Virus Serotype 1 (MDV1) Glycoprotein D in MDV1-Infected Chick Embryo Fibroblasts

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ABSTRACT. Chick embryo fibroblasts (CEFs) infected with three strains of Marek's disease virus serotype 1 (MDV1), GA, Md5 and JM, were subjected to indirect immunofluorescence assay with monoclonal antibodies (MAbs) against MDV1 homolog of glycoprotein D (MDV1 gD) of herpes simplex virus. By the MAbs, a number of MDV1 gD-positive cells were detected in CEFs infected with GA, whereas only a few and no positive cells were detected in CEFs infected with Md5 and JM, respectively. The MDV1 gD in GA-infected CEFs was recognized as the band of 64 kDa in immunoblot analysis using one of the MAbs. This is the first report that the MDV1 gD was detected in MDV1-infected cell cultures. — KEY WORDS: glycoprotein D, Marek's disease virus serotype 1.

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Marek's disease virus (MDV) serotype 1 (MDV1) is a causative agent of Marek's disease (MD), a lymphoproliferative disease of chickens. MDV1 spreads in a cell-associated form and cell-free viruses can be obtained only from feather follicle epithelium. MD is controlled by vaccination with attenuated MDV1, nonpathogenic MDV serotype 2 (MDV2) or serotype 3 (herpesvirus of turkeys [HVT]). All three MDV serotypes resemble alphaherpesviruses in genomic structure and gene arrangement [4, 5, 8, 11, 19]. Envelope glycoproteins of alphaherpesviruses have major roles in the infectious process and are predominant targets of both humoral and cell-mediated immunity. Therefore, it is important to analyze the glycoproteins of MDV1. Two MDV1 glycoproteins (MDV1 gB and MDV1 gC), MDV1 homologs of herpes simplex virus (HSV) glycoproteins B and C were well characterized. The homologs of glycoprotein B of alphaherpesviruses are essential for virus replication [25]. MDV gB is considered to play an important role in virus infectivity because the MDV1 gB elicited neutralizing antibodies [13, 17]. MDV1 gC is present in both culture fluids and cell extracts of MDV1-infected cells. MDV1 gC is considered to be nonessential for virus replication *in vitro* because the amount of MDV1 gC is reduced during serial passages in cell cultures [12, 18]. HSV glycoprotein D (gD) and its homologs of pseudorabies virus (PRV) gp50 and bovine herpesvirus type 1 (BHV-1) glycoprotein IV (gIV) are essential for virus penetration in cell cultures [7, 15, 22, 23]. PRV gp50 is dispensable for direct cell-to-cell transmission both in cell cultures and in animals [10]. On the other hand, varicella-zoster virus genome does not encode gD homologous gene [6].

In MDV1 and HVT genomes, the potential open reading frames (ORFs) which are homologous to the HSV gD gene were found [2, 24, 28]. However, the gD homolog of MDV1 (MDV1 gD) seems to be nonessential for virus growth in cultured cells and chickens, because a mutant MDV1 whose MDV1 gD gene was disrupted by insertion of *lacZ* gene of *Escherichia coli* could replicate in chick embryo fibroblasts (CEFs) and in chickens [21]. Furthermore, Brunovskis and his colleagues reported that MDV1 gD was not detected in MDV1-infected duck embryo fibroblasts (DEFs) by immunoprecipitation with antisera against MDV1 gD-trpE fusion protein [3]. However, further studies should be required to clear functions of the MDV1 gD, because gD plays an important role in virus infection and penetration to target cells, and protection in other alphaherpesviruses. To analysis MDV1 gD, we previously constructed the recombinant baculovirus which expressed MDV1 gD and prepared monoclonal antibodies (MAbs) which recognized the recombinant MDV1 gD [20]. In this paper, we examined expression of the MDV1 gD in CEFs infected with three strains of MDV1, GA, JM and Md5, using the MAbs.

The three strains of MDV1 used in this study were kindly provided by Dr. K. Imai (National Institute of Animal Health, Japan). Passage levels of the strains before inoculation in chickens are not certain. These strains had been inoculated into chickens, reisolated from bloods of the chickens, and then passed four or five times in CEFs. Mock-infected CEFs or those infected with one of the three strains of MDV1 were fixed in ice-cold acetone and subjected to immunofluorescence assay (IFA) using five anti-MDV1 gD MAbs, 5B1, 6F11, 10D3, 11A12, and 11F10 [20], anti-MDV1 gC MAb, M26 [12, 18], and anti-MDV1 gB MAb, M51 [13, 17]. The three anti-MDV1 gD MAbs recognizes different epitopes [20].

The results of IFA on GA-infected CEFs are shown in Fig. 1. GA-infected CEFs stained by anti-MDV1 gD MAbs showed fluorescence (a result using 6F11 is shown in Fig. 1A), whereas mock-infected CEFs stained by the MAbs did not (Fig. 1B). GA-infected CEFs stained with M26 (anti-MDV1 gC) and M51 (anti-MDV1 gB) also showed fluorescence (Fig. 1C and 1D, respectively), and similar results were obtained in IFA on JM- and Md5-infected CEFs

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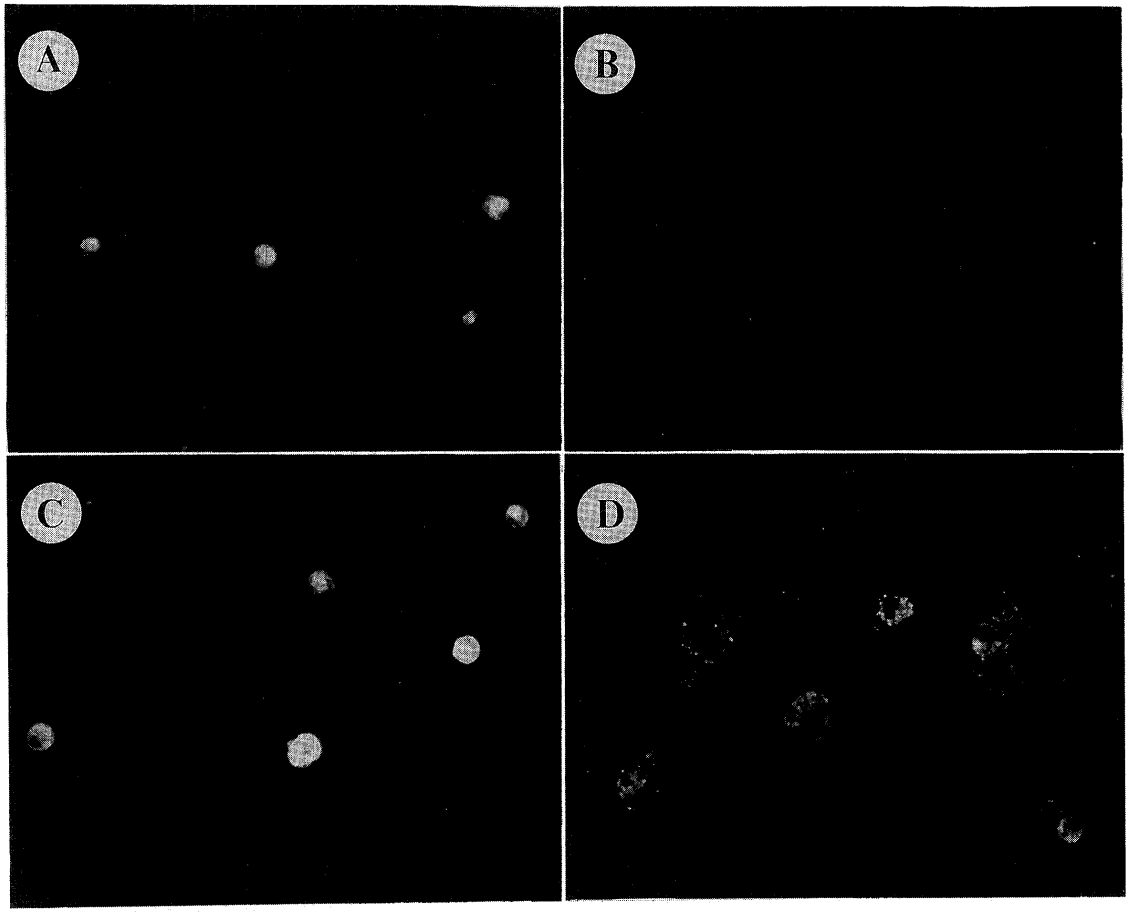


Fig. 1. Immunofluorescence analyses of GA- or mock-infected CEFs with MAbs 6F11 (A or B, respectively), and GA-infected CEFs with M26 (C), and M51 (D). × 200.

by M26 and M51 (data not shown). As shown in Table 1, the percentages of positive cells in IFA were counted in three random-sampled view fields of CEFs infected with JM, Md5 or GA strain using M51, M26 and 6F11. Results using the other anti-MDV1 gD MAbs were similar to those using 6F11. Mock-infected CEFs stained with all of the MAbs used did not show any fluorescence (data not shown). CEFs infected with the three strains were similar in percentages of positive cells when stained with M51, suggesting that the percentages of cells infected with the three strains should be similar. On the other hand, CEFs infected with the three strains were different in percentages of positive cells when stained with M26, suggesting that levels of reduced expression of the MDV1 gC were different among the three strains.

To characterize the antigen which was recognized in IFA on GA-infected CEFs by 6F11, we examined the molecular weight of the antigen by immunoblot analysis using 6F11. The antigen was detected as a band of 64 kDa (Fig. 2 lane 4), whereas the recombinant MDV1 gD expressed by the recombinant baculovirus was detected as a broad band ranging from 49 to 52 kDa (Fig. 2 lane 2). This band must consist of three bands of 49, 50, and 52 KDa as described

Table 1. Percentages of cells displaying positive staining in immunofluorescence assay

| MDV strain | Monoclonal antibody | | |
|---|---|---|---|
| | M51 | M26 | 6F11 |
| JM | 34 ± 3.7[a)] | 6.9 ± 1.4 | 0 ± 0.0 |
| Md5 | 37 ± 3.0 | 1.9 ± 0.0 | 0.3 ± 0.3 |
| GA | 40 ± 2.2 | 5.5 ± 0.0 | 5.8 ± 1.6 |

a) Standard deviation.

previously [20]. A band of 97 kDa was also detected in the recombinant baculovirus-infected cell lysate. This band is considered to be a dimer of the recombinant protein. In other alphaherpesviruses, the authentic HSV gD, BHV-1 gIV, and equine herpesvirus gD were also larger than the recombinant proteins expressed by the baculoviruses [9, 14, 16, 26]. The differences seemed to be caused by the level of glycosylation [1].

GA-infected CEFs stained by anti-MDV1 gD MAbs did show some fluorescence in this study but Brunovskis and his colleagues reported that the MDV1 gD was not detected in GA-infected DEFs by immunoprecipitation with antisera

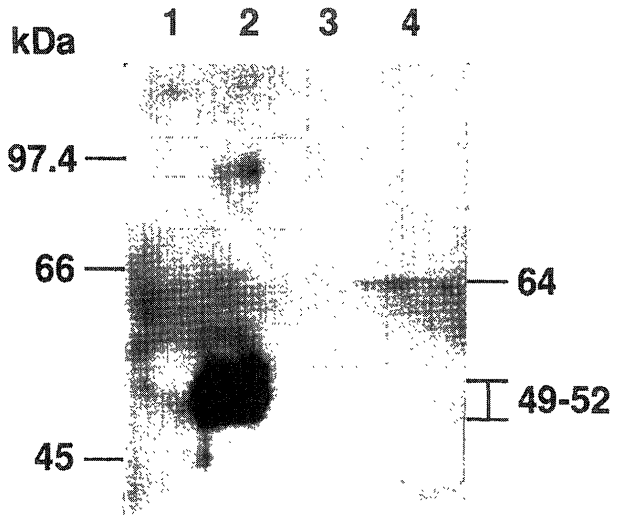


Fig. 2. Immunoblot analysis of MDV1 gD under reducing conditions. Lysate of *Spodoptera frugiperda* cells infected with the recombinant baculovirus, cAcNPV (lane 1) or rAcMDV1gD (lane 2), and lysate of CEFs infected with mock (lane 3) or GA (lane 4) were probed with the anti-MDV1 gD MAb, 6F11 and peroxidase-conjugated anti-mouse IgG, and then visualized using ECL Western blotting detection reagents (Amersham international plc, Buckminghamshire, U.K.). The rAcMDV1gD is the recombinant baculovirus which expresses the recombinant MDV1 gD and cAcNPV is the baculovirus which does not carry any foreign genes [20].

against MDV1 gD-trpE fusion protein [3]. The contrast results might be due to the differences in sensitivities of the methods or antibodies used. On the other hand, JM-infected CEFs stained by the anti-MDV1 gD MAbs did not show any fluorescence. The anti-MDV1 gD MAbs used in this study were prepared using the recombinant protein expressed from MDV1 gD ORF of JM as an antigen [20]. Therefore, it is suggested that JM-infected CEFs did not express the MDV1 gD. Difference in the expression of MDV1 gD among the three strains is of interest. By sequence analysis, the potential ORF encoding MDV1 gD was found in the genomes of RB1B and GA strains [2, 24]. The potential ORF was probably conserved in the genome of JM because the recombinant baculovirus which carried the DNA fragment of JM possibly including MDV1 gD ORF expressed the recombinant protein whose size is reasonable to consider that the protein was generated from the MDV1 gD ORF [20]. Therefore, it is considered that the difference in expression of MDV1 gD should result from differences in process of transcription and/or translation. In IFA on GA-infected CEFs, the percentage of positive cells when stained with M26 or 6F11 was smaller than that with M51. Wilson and her colleagues suggested that the reduced expression of the MDV1 gC resulted from alteration of MDV regulatory protein(s) which interacts with the MDV1 gC promoter [27]. The reduced expression of the MDV1 gD is considered to depend on different factors because the percentage of positive cells in IFA on JM-infected CEFs when stained with 6F11 was different from that with M26. Further studies should be required to solve this subject.

The role of the MDV1 gD in the pathogenesis of MDV1 infection still remains unclear. However, the two strains, GA and JM, which are different in the expression of the MDV1 gD, and the MAbs which could detect the expression of MDV1 gD should be useful for further studies.

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