A MONOCLONAL ANTIBODY TO ICP4 OF MDV RECOGNIZING ICP4 OF SEROTYPE 1 AND 3 MDV STRAINS

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Summary. – Monoclonal antibodies (MAbs) were prepared against ICP4 of Marek's disease virus (MDV). Mice were inoculated with ICP4 obtained from High-Five insect cells infected with a recombinant baculovirus expressing ICP4. MAbs were selected by enzyme-linked immunosorbent assay (ELISA) using MDV-infected and control chick kidney cells as antigens. One of the MAbs, 5H8, recognized an epitope toward the carboxyl terminus of ICP4 based on staining of reticuloendotheliosis virus-transformed cells transfected with full-length and truncated ICP4 constructs. This MAb recognized ICP4 in chicken embryo fibroblasts (CEFs) infected with MDV strains JM16 and HVT but not with SB-1 strain. Using Western blot analysis a protein of 155 kDa was detected in CEFs infected with JM16 and HVT strains.

Key words: MDV; ICP4; monoclonal antibody

Introduction

MDV is a highly cell-associated and oncogenic herpesvirus causing T cell lymphomas in chickens (Calnek and Witter, 1997). Based on serology, protein structure and genetic analysis, MDV has been divided into three serotypes. MDV serotype 1 (MDV-1) consists of all oncogenic and attenuated strains (e.g., RB-1B, JM16 and GA-5), MDV serotype 2 (MDV-2) consists of naturally occurring nononcogenic strains (e.g., SB-1), and MDV serotype 3 (MDV-3) consists of nonpathogenic herpesvirus of turkeys (HVT) strains (Bülow and Biggs, 1975; Lee *et al.*, 1983; Ross *et al.*, 1983; Schat, 1985).

The genomic structure of MDV is closely related to that of alpha-herpesviruses (e.g., herpes simplex virus (HSV)). MDV DNA is approximately 180 kbp in length and consists of unique long (U_L) and a unique short (U_S) regions which are flanked by sets of inverted repeats: long terminal repeat (TR_L), long internal repeat (IR_L), short terminal repeat (TR_S) and short internal repeat (IR_S), (Buckmaster *et al.*, 1988). Although the complete sequence of MDV has not yet been reported, several genes have been mapped. The gene for the ICP4 homologue of MDV-1 has been mapped to the *Bam*HI-A fragment and is located within the IR_S and TR_S. The predicted coding region of MDV ICP4 consists of 4245 nt encoding a protein of 155 kDa. The overall structure of the protein is similar to that of HSV and other alpha-herpesviruses (Anderson *et al.*, 1992). ICP4 is essential in alpha-herpesviruses for the induction of viral DNA synthesis and transactivation of the transcription of early and late genes. ICP4 can also downregulate its own gene expression. Overexpression of ICP4 in the MDCC-MSB-1 cell line resulted in increased transcription and expression of pp38 (Pratt *et al.*, 1984). Zelník *et al.* (1996) reported recently that the overall structure of ICP4 of HVT is similar to that of MDV-1.

MAbs have been used extensively for the characterization of MDV proteins. Serotype-specific MAbs were first developed by Lee *et al.* (1983). In this report, we describe the development of an ICP4-specific MAb using ICP4 purified from a recombinant baculovirus as a source of antigen. This MAb detected an epitope shared by MDV-1 and MDV-3.

Materials and Methods

Viruses. Autographa californica nuclear polyhedrosis virus (AcMNPV), a member of the *Baculoviridae* family, was described previously (Smith *et al.*, 1985; Summers and Smith, 1987).

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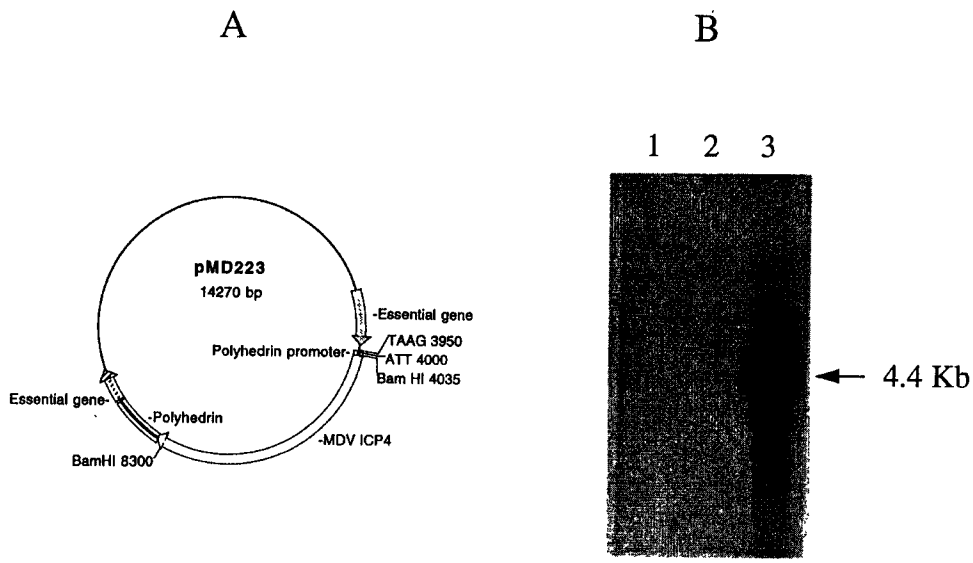

Fig. 1
Construction of recombinant ICP4 baculovirus (A) and its Southern blot analysis (B)
(A) The 4.37 kbp *Bam*HI fragment containing the MDV ICP4 coding region was purified from plasmid pMD145 and integrated into the pVL941 AcMNPV transfer vector to generate pMD223. (B) Southern blot analysis of putative recombinant AcMNPV DNA digested with *Bam*HI and hybridized with the 4.37 kbp *Bam*HI fragment from pMD145. DNA from uninfected SF9 insect cells (lane 1); DNA from wild-type AcMVPV-infected cells (lane 2); DNA from putative recombinant baculovirus-infected SF9 cells (lane 3).

Oncogenic MDV-1, JM16 strain passage 19 (JM16/19) and its attenuated form, JM16 strain passage 46 (JM16/46), MDV-2 SB-1 strain and HVT-3 were propagated in chicken kidney cells (CKCs) and CEFs, respectively, as previously described (Schat *et al.*, 1982).

Plasmid construction. The construction of recombinant pVL941-ICP4 is shown in Fig. 1. The pVL941 vector contains the entire AcMNPV nuclear polyhedrin gene locus cloned into the pUC8 vector. The polyhedrin gene locus contains the upstream promoter, gene codon region and polyadenylation site. A 4.37 kbp *Bam*HI fragment containing the entire ICP4 coding region was prepared from plasmid pMD145 (Anderson *et al.*, 1992), ligated into the *Bam*HI-digested pVL941 (Pharmingen, San Diego, CA) and the resulting plasmid was named pMD223 (Fig. 1A).

For expression of ICP4 in lymphoblastoid cell lines, full-length and truncated fragments of the MDV ICP4 gene were cloned into the expression vector pcDNA3.1 downstream of the immediate early CMV promoter (Invitrogen). The 3'-truncated 3.0 kbp and 1.6 kbp fragments were prepared from pRC/CMV-ICP4 (Omar and Schat, 1996) by endonuclease digestion with *Not*I-*Spe*I and *Not*I-*Bst*1107 to generate 3 kbp and 1.6 kbp fragments, respectively. These fragments were cloned into pcDNA3.1 to obtain expression vectors pcDNA3.1-ICP4/3.0 and pcDNA3.1-ICP4/1.6, respectively.

Transfection procedures. Spodoptera frugiperda (Sf9) cells were maintained in TNM-FH medium (GIBCO BRL) supplemented with 10% fetal calf serum (GIBCO) and were transfected with 0.15 μg of linearized BaculoGold DNA and 1.5 μg of pMD223 by using the BaculoGold Transfection Kit (Pharmingen) as described (Smith *et al.*, 1985). Recombinant AcMNPVs (rICP4-AcMNPVs) were purified nine days later after plaques were visualized in Sf9 cells and amplified in High-Five (Invitrogen) insect cells maintained in Grace's Insect Medium (GIBCO) with 10% FBS.

Plasmids for transfection of reticuloendotheliosis virus (REV)-transformed lymphoblastoid cell lines were prepared using the QIAGEN Plasmid Midi Kit (Qiagen Inc.). Transfection of REV-transformed lymphoblastoid cell lines was performed as previously described (Omar and Schat, 1996). Briefly, 5 x 10^6 REV-transformed lymphoblastoid cells, CU91 ($B^{19}B^{19}$) and CU205 ($B^{21}B^{21}$), after a Ficoll-Hypaque gradient centrifugation (Pharmacia) were resuspended in 0.4 ml of LM Base Medium (Schat *et al.*, 1992) with 5 μg of linearized plasmid in a 0.4-cm sterilized cuvette, and transfected by electroporation using the Gene Pulser Apparatus (Bio-Rad) set at 300 V with capacitance extender set at 500 μF. Afterwards, the cells were left at room temperature for 5 mins, resuspended in 4 ml of LM Base Medium supplemented with 10% FBS (LM10) (GIBCO) and incubated at 41°C. Forty-eight hours after transfection, medium was changed to LM10 containing 1000 to 1200 μg/ml of active G418 (Life Technologies) and maintained at 41°C with medium changes at 48 to 72 hrs intervals for 3 to 4 weeks until G418-resistant groups of cells were detected. Individual groups were selected for cloning by limited dilution in the presence of G418. Cloned, stably transfected cell lines were maintained in LM10 with 300 μg of active G418.

Southern blot and Northern blot analyses. Two μg of total DNA from AcMNPV-infected or rICP4 AcMNPV-infected Sf9 cells was digested with *Bam*HI, and the resulting DNA fragments were se-

parated in 0.8% agarose gel and transferred to nitrocellulose. The blot was probed with the 4.37 kbp *Bam*HI fragment from pMD145 (Anderson *et al.*, 1992). For Northern blot analysis, High-five cells were infected with AcMNPV or rAcMNPV for 48 hrs. Then total RNA was purified from the cultures using the guanidinium isothiocyanate method and 5 µg of total RNA from each sample was used for Northern blot analysis using the standard protocol. A riboprobe was generated from the ICP4 fragment using [α-^{32}P]CTP and a commercial *in vitro* transcription kit (Promega).

Reverse transcription-polymerase chain reaction (RT-PCR). Total cellular RNAs from nontransfected and transfected REV-transformed lymphoblastoid cells were isolated using the Micro RNA Isolation Kit (Stratagene). RNA samples were ethanol-precipitated, washed with 75% ethanol, reconstituted in diethylpyrocarbonate (DEPC)-treated water, and the RNA concentrations were determined by measuring the A_{260} value. Aliquots of the RNA preparations were stored at -70°C. Approximately 2 µg of RNA samples was used in RT-PCR using the GeneAmp RNA PCR Kit (Perkin-Elmer), following the protocol provided by the manufacturer. Samples were also amplified without the RT step to demonstrate the absence of DNA contamination. Upstream and downstream oligonucleotide primers, complementary to the internal coding region of the 5'-region of ICP4 (5'-CTTTATGCGACTGGCGTGCTGCCGTA-3' and 5'-TCCTCC TGCACGCCTGTTTGCGGT-3'), were used. RT-PCR products were resolved in 1 to 1.2% agarose gel (SeaKem LE, FMC).

Preparation of purified ICP4 and immunization of Balb/c mice. High-Five cells were infected with rICP4AcMNPV or wild type AcMNPV for 48 hrs. Infected cells were washed twice with cold TD buffer (25 mmol/l Tris, 136 mmol/l NaCl, 5.7 mmol/l KCl, and 7 mmol/l Na_2HPO_4, pH 7.4) and removed from the plates in cold TD buffer by scraping with a rubber policeman. Cells were pelleted and incubated in lysis buffer (150 mmol/l Tris pH 7.4, 150 mmol/l NaCl, 1 mmol/l EDTA, 10% glycerol, 10% NP-40, 1 mmol/l phenylmethylsulfonyl fluoride, 2 µg/ml aprotinin proteinase inhibitors (Sigma)) on ice for 30 mins. Cell lysates were pelleted at 14,000 rpm in a microcentrifuge. Proteins in the supernatant fluid were separated using a preparatory 7.5% plyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE). The location of the nonstained ICP4 protein band was based on a part of the gel stained with Coomassie Brilliant Blue (FisherBiotech). The fragment containing the 155 kDa protein band was removed from the gel, and proteins were electrophoretically eluted using Tris-glycine electrophoresis buffer (25 mmol/l Tris, 250 mmol/l glycine (electrophoresis grade) pH 8.3, and 0.1% SDS) and concentrated 6-fold using PEG 30,000. Purified protein was used to immunize two Balb/c mice a total of 4 times with 300 µg protein per injection. The fourth immunization was given one week before spleen cells were harvested.

Fusion of hybridoma cells and screening of MAbs. The fusion of myeloma and spleen cells from immunized mice was carried out following established procedures. Briefly, 50 x 10^6 SP6/0 myeloma cells were mixed with 10^8 spleen cells from one mouse in cold DMEM without serum, centrifuged for 10 mins at 400 x g, the supernatant fluid was removed, and the cells were treated with polyethylene glycol (PEG 1400) in a water bath at 37°C. After the PEG treatment, DMEM was added dropwise to the mixture, the cells were incubated for 5 mins at 37°C, and centrifuged. After removing the supernatant fluid the cells were resuspended in 100 ml of 2 x HAT solution (DMEM with 20% FBS), seeded into 96-well plates and incubated at 37°C with 5% CO_2.

Supernatant fluids were tested for the presence of MAbs using an immuno-peroxidase monolayer assay essentially as described by Peeters *et al.* (1992) with some modifications. Briefly, 96-well plates containing CKC cultures were infected with MDV JM16/p19 and fixed with methanol/ethanol (1:1) 3 days post infection. Uninfected CKC plates were used as negative controls. Hybridoma supernatant fluids were added to the wells, incubated for 30 mins at 37°C. The plates were washed three times with PBS containing Tween-80 (0.05%). Affinity-purified rabbit anti-mouse IgG+A+M (H+L)-specific antibody, conjugated with highly purified HRP, diluted at 1:4000 in PBS containing 1 % BSA was added, the plates were incubated for 30 mins at 37°C, and washed five times with Tween 80-PBS. An ABTS Solution Substrate Kit (Zymed) was used to detect the complex of the HRP-conjugated antibody and ICP4 MAbs. Wells with cells producing positive supernatant fluids were cloned three times by limited dilution.

Indirect immunofluorescence assay (IFA) was used to detect ICP4 antigens in MDV-infected CEFs and transfected lymphoblastoid cells expressing ICP4. Serotype-specific MAbs were kindly provided by Dr. L.F. Lee (Avian Disease and Oncology Laboratory, East Lansing, MI). IFA cells were fixed in a mixture of acetone/methanol (1:1) and incubated with MAbs for 30 mins at 37°C, washed in PBS (pH 7.2) for 15 mins, incubated with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse antibodies (Southern Biotechnologies) for 30 mins at 37°C and washed in PBS for 15 mins. Cells were examined with a Leitz Dialux fluorescence microscope equipped with epi-illumination.

Western blot analysis was performed (a) to identify the recombinant protein in High-Five cells infected with wild type AcMNPV, or rAcMNPVs expressing ICP4 or SV40 T-antigen for 48 hrs and (b) to demonstrate the presence of ICP4 in MDV-infected cells. (a) High-Five cells were washed twice with cold TD buffer (25 mmol/l Tris, 136 mmol/l NaCl, 5.7 mmol/l KCl, and 7 mmol/l Na_2HPO_4 pH 7.4) and removed from the plates in cold TD buffer by scraping with a rubber policeman. Cells were recovered by centrifugation in a microcentrifuge and the cell pellets were treated for 30 mins on ice with lysis buffer (150 mmol/l Tris pH 7.4, 150 mmol/l NaCl, 1 mmo/l EDTA, 10% glycerol, and 10% NP-40) containing 1 mmol/l phenylmethylsulfonyl fluoride (PMSF), 100 µg/ml tosylphenylalanine chloromethyl ketone (TPCK), 50 µg/ml tosyllysine chloromethyl ketone (TLCK) and 2 µg/ml aprotinin proteinase inhibitors. Cell lysates were spun at 14,000 rpm in a microcentrifuge. For Western blot analysis, cell lysates were separated by SDS-PAGE and blotted to nitrocellulose membranes for 2 hrs at 150 mA using an electrotransfer apparatus (Bio-Rad). After treatment with blocking buffer (5% dry skim milk, 0.3% Tween in Tris-buffered saline [TBS]) for one hour, the blots were incubated for one hour with anti-MBP-ICP4 antibody (Xie, unpublished data) diluted 1:500 in blocking buffer. The blots were washed three times with TBS and incubated for one hour with anti-rabbit alkaline phosphatase-conjugated goat antiserum (Sigma) diluted 1:1000 in blocking buffer. After three additional washes with TBS, the blots were incubated with substrate solution containing 0.5 mg of 5-bromo, 4-chloro, 3-indolyl phosphate and 1 mg of nitro blue tetrazolium in 10 ml of 100 mmol/l Tris pH 9.5, 100 mmol/l NaCl and 5 mmol/l $MgCl_2$ until bands were apparent. (b) CEFs infected

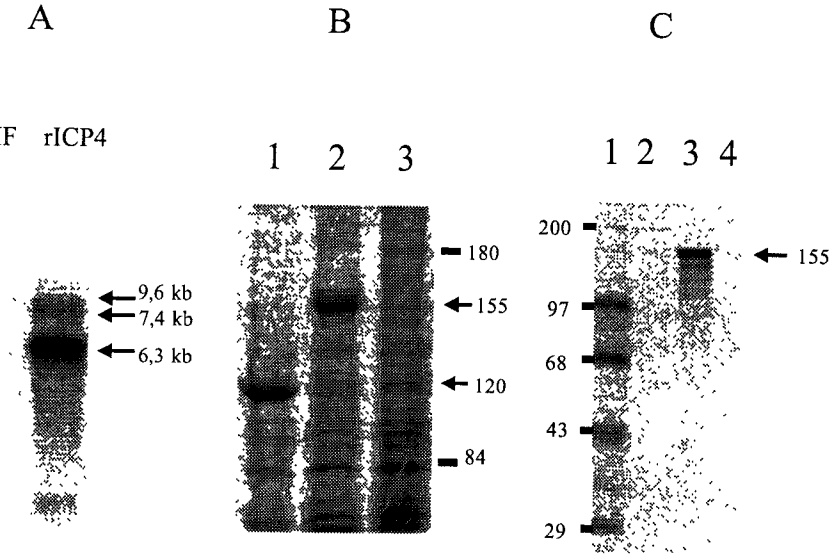

Fig. 2
Expression of ICP4 RNA and protein in High-Five cells infected with rICP4AcMNPV
(A) Northern blot analysis of putative recombinant baculovirus. RNA from uninfected High-Five cells (lane HF); RNA from rICP4AcMNPV-infected High-five cells (lane rICP4). The RNA was hybridized to the ICP4 riboprobe generated from pMD201. (B) SDS-PAGE. Recombinant AcMNPV expressing 120 kDa β-galactosidase (lane 1); lysate of rICP4AcMNPV-infected cells (lane 2); lysate of mock-infected cells (lane 3). (C) Immunoprecipitation analysis. Size markers (lane 1); lysate from rTAg-AcMNPV-infected cells (lane 2); lysate from rICP4AcMNPV-infected cells (lane 3); lysate from wild-type AcMNPV-infected cells (lane 4).

with JM16, SB-1 and HVT, and uninfected cells were harvested 3 to 4 days post infection. Infected and non-infected cells were lysed in a lysis buffer (1% NP-40, 20 mmol/l Tris-HCl pH 7.4, 150 mmol/l NaCl, 2.5 mmol/l EDTA, 0.1% SDS, 20 ml/ml leupeptin and 1 mmol/l phenylmethylsulfonyl fluoride) on ice and clarified by centrifugation. Cell lysates (20 μg) were resolved by SDS-PAGE and proteins were transferred to nitrocellulose membranes as described and treated with blocking buffer. The blots were incubated with MAb 5H8 diluted 1:2000 in TBST (50 mmol/l Tris-HCl pH 7.4, 150 mmol/l NaCl and 0.05% Tween-20) for 1.5 hr at room temperature. After washing four times in TBST, the blots were incubated with HRP-conjugated rabbit-anti-mouse antibodies (Amersham Life Science) for 1.5 hr, subjected to a chemiluminiscence substrate reaction system (ECL, Amersham), washed five times with TBST, and finally autoradiographed.

Radio-immunoprecipitation. High-Five cells were infected with wild type AcMNPV or rAcMNPVs expressing ICP4 or SV40 T-antigen for 48 hrs. Infected cells were washed with Hanks buffered saline solution three times and incubated for 1 hr in labelling medium containing methionine-free Graces medium supplemented with 5% dialyzed FBS and containing 50 μCi ^{35}S-methionine per ml. After 4 hrs, cells were lysed as described before. The supernatant was collected and 5 μl of normal rabbit serum was added to the supernatant at 4°C overnight. Fifty μl of the Cowan strain of *Staphylococcus aureus* (StaphA) was added to the cell lysate. After incubation at 4°C for 1 hr, the lysate was centrifuged and the supernatant was collected. Five μl of anti-MBP-ICP4 polyclonal antibody was added to the supernatant followed by incubating the supernatant at 4°C for 2 hrs. Fifty μl of Staph A was added to the supernatant and the mixtures were incubated at 4°C for 1 hr. Immunoprecipitates were then washed once with each of the following reagents: lysis buffer, lysis buffer containing 0.5 mol/l LiCl, and lysis buffer. Subsequently, immunoprecipitates were suspended in the sample buffer (50 mmol/l Tris pH 6.8, 2% SDS, 0.1% Bromophenol Blue, 10% glycerol and 1% mercaptoethanol) and incubated at 65°C for 25 mins. After an additional centrifugation for one minute at room temperature, the supernatant was analyzed by SDS-PAGE.

Results

Construction and selection of recombinant ICP4 baculovirus

Recombinant AcMNPV was generated by cotransfecting Sf9 cells with linearized BaculoGold DNA and pMD223. DNA purified from each putative recombinant AcMNPV plaque was examined by PCR using ICP4 internal primers confirming that the recombinant virus contained the ICP4 gene (data not shown). *Bam*HI-digested DNA purified from rICP4-infected but not from Sf9 cells infected with wild-type virus or mock-infected cells hybridized with the 4.37 kbp ICP4-specific probe showing the expected 4.4 kbp band (Fig. 1B).

Expression of ICP4 in High-Five cells infected with rICP4AcMNPV

The transcription of the ICP4 gene in rICP4AcMNPV was examined by Northern blot analysis using total RNA obtained from mock-infected or rICP4AcMNPV-infected High-Five cells at 48 hrs post infection. Three ICP4-specific transcripts of 9.6 kb, 7.4 kb and 6.3 kb were detected (Fig. 2A) after hybridization with the ICP4-specific riboprobe. A band at approximately 155 kDa was detected by SDS-PAGE (Fig. 2B). A single band of 155 kDa was detected by radio-immunoprecipitation using the rabbit anti-ICP4 serum (Fig. 2C).

Fusion and selection of hybridoma cells secreting ICP4 MAbs

Hybridoma clones became visible two weeks after fusion. Supernatant fluids of 32 wells were positive using CKCs infected with JM16/19. These supernatant fluids did not react with uninfected CKCs. Cells from six positive wells were selected for two rounds of limited dilution cloning and were subsequently amplified in 25 cm^2 flasks. Ascitic fluid of clone 5H8 was used for subsequent experiments.

IFA results

MDV JM16-infected CEFs but not uninfected CEFs were positive in the IFA using MAb 5H8 (Fig. 3B), while ascitic fluid from an unrelated MAb did not stain these cells (Fig. 3C). The IFA staining with 5H8 was relatively weak when compared to the staining with a MAb against glycoprotein B (Fig. 3A). MAb 5H8 reacted also with HTV-infected but not with SB-1-infected CEFs (Fig. 4).

Immunofluorescence staining of ICP4-transfected lymphoblastoid cells

Lymphoblastoid cell lines stably expressing full-length or the 1.6 or 3.0 kbp truncated ICP4 genes were established by transfecting REV cell lines CU91 and CU205 (Fig. 5). These cell lines expressed ICP4 transcripts as demonstrated by RT-PCR (Fig. 6). MAb 5H8 was used in an IFA to detect the presence of ICP4 proteins in the transfected lymphoblastoid cell lines. Only the cell line expressing full-length ICP4 was positive in this assay.

Western blot analysis of MDV-infected cells using MAb 5H8

Cell lysates from CEFs infected with MDV strains JM16, SB-1 and HVT, were resolved by SDS-PAGE, and proteins were blotted to a nitrocellulose membrane, and incubated with MAb 5H8. An 155 kDa band was detected in the lysates from JM16- and HVT-infected but not SB-1 infected cells or uninfected control cells (Fig. 7).

Discussion

In this report, we used MDV ICP4 expressed from a recombinant baculovirus to immunize mice to generate MAbs to ICP4. One of the MAbs (5H8) was further characterized. Western blot analysis indicated that 5H8 detected a protein of approximately 155 kDa in lysates from CEFs infected with MDV-1 and MDV-3 strains. Recently Zelník *et al.* (1996) reported sequencing of the *Bam*HI-A fragment of HVT, and analysis of HVT IR_S/TR_S sequence revealed the presence of a large ORF. Its C-terminal part displays homology to ICP4 proteins of alpha-herpesviruses. Further study showed that extension at its N-terminus encoding 800 amino acids exhibits in certain regions significant homology to the upstream encoded sequence of MDV-1 ICP4. The positive staining of REV cell lines transfected with full-length ICP4 but not with the truncated constructs suggests that the epitope of ICP4 in MDV-1 is located, at least partially, in the C-terminal 400 amino acids of ICP4. These data support the hypothesis that the epitope in MDV-1 and HVT is located in the C terminus of MDV-1 and MDV-3. The finding that MAb 5H8 did not detect the presence of ICP4 in SB-1, a serotype 2 strain, suggests that serotype 1 and 3 strains are genetically more related to each other than to serotype 2 strains. This hypothesis is compatible with the observation that antibodies against MDV-1 gB can neutralize HVT but not MDV-2 strains (Ikuta *et al.*, 1984). It is also in agreement with the finding that MAb IAN86 recognizing a discontinuous virus neutralizing epitope of serotype 1 gB (Yanagida *et al.*, 1992) can also recognize gB of serotype 3 but not of serotype 2 strains (Yoshida *et al.*, 1994).

To generate a recombinant baculovirus expressing ICP4, a 4.37 kbp ICP4 gene, which contains the predicted translational start site ATG and polyA signal, was inserted into the transfer vector under the control of the polyhedrin promoter. However, in the Northern blot analysis, the expected 4.22 kb MDV ICP4 transcript was not detected. Instead, a major 6.3 kb RNA and two minor 7.4 kb and 9.6 kb RNAs were detected in rICP4AcMNPV-infected High-Five cells. It is likely that the 6.3 kb RNA was derived from the MDV ICP4 gene and the downstream polyhedrin gene. The 9.6 kb and 7.4 kb RNAs were likely derived from the MDV ICP4 gene, the polyhedrin gene, and genes downstream of the polyhedrin gene. It is possible that RNA polymerase II passed through the polyA signal located right after the predicted ICP4 coding region terminating transcription at the polyA signal of the polyhedrin gene and further downstream. This result is consistent with the ICP4 RNA mapping study (unpublished data) in which riboprobes generated from the

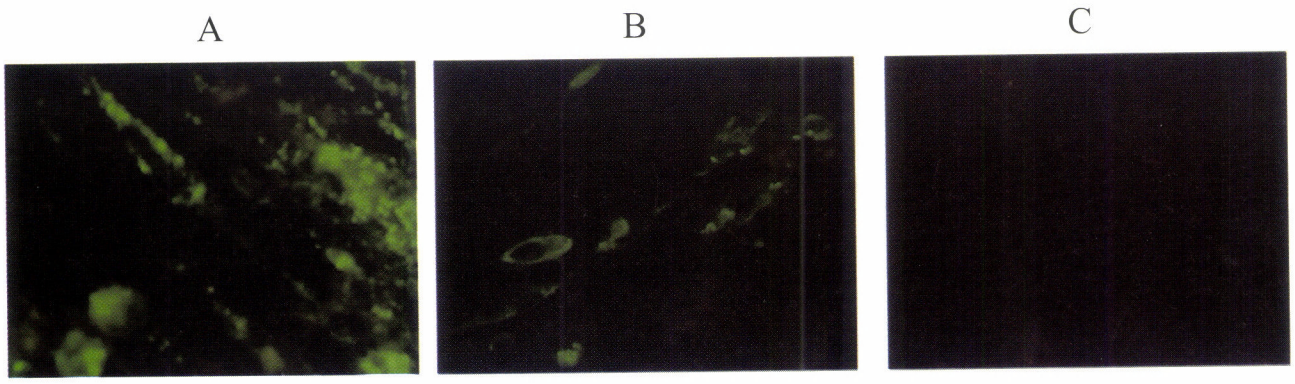

Fig. 3
Indirect immunofluorescence staining of MDV JM16-infected CEFs using different MAbs
(A) gB-specific MAb, (B) ICP4-specific MAb (5H8), (C) unrelated MAb.

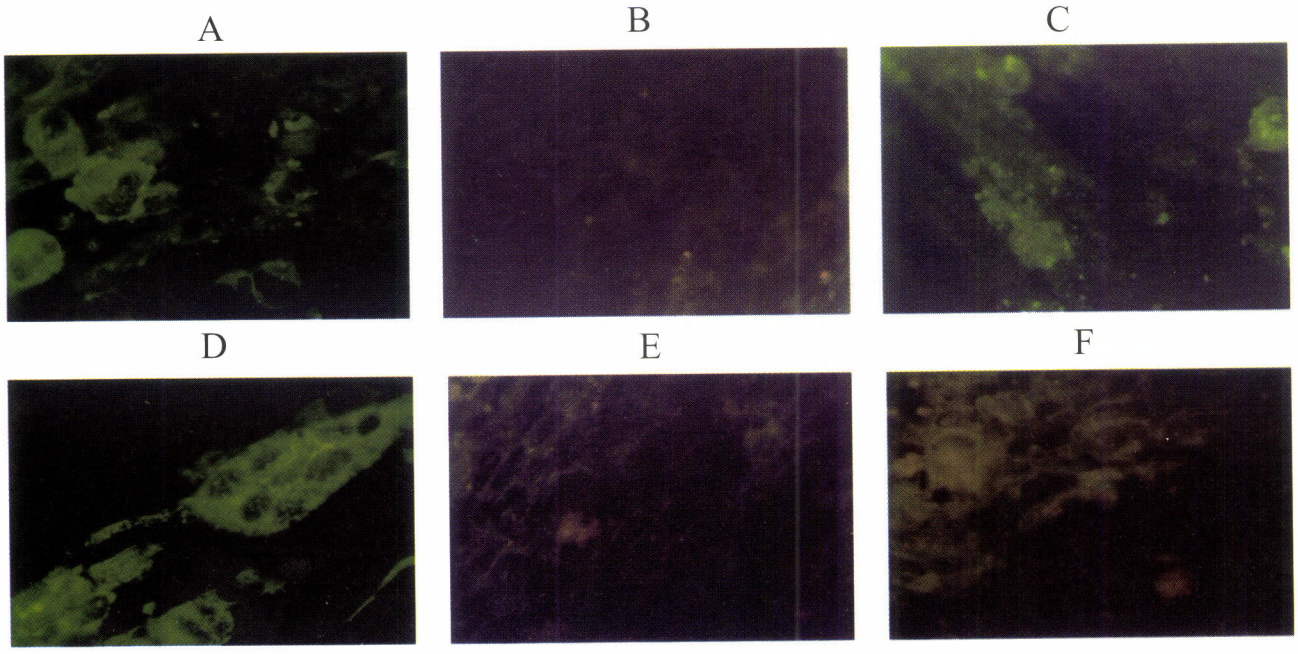

Fig. 4
Immunofluorescence staining of CEFs infected with MDV strains JM16, SB-1 and HVT
(A) JM-16, MAb 5H8; (B) SB-1, MAb 5H8; (C) HVT, MAb 5H8; (D) SB-1, MDV-2-specific MAb; (E) JM16, unrelated MAb; (F) HVT, unrelated MAb.

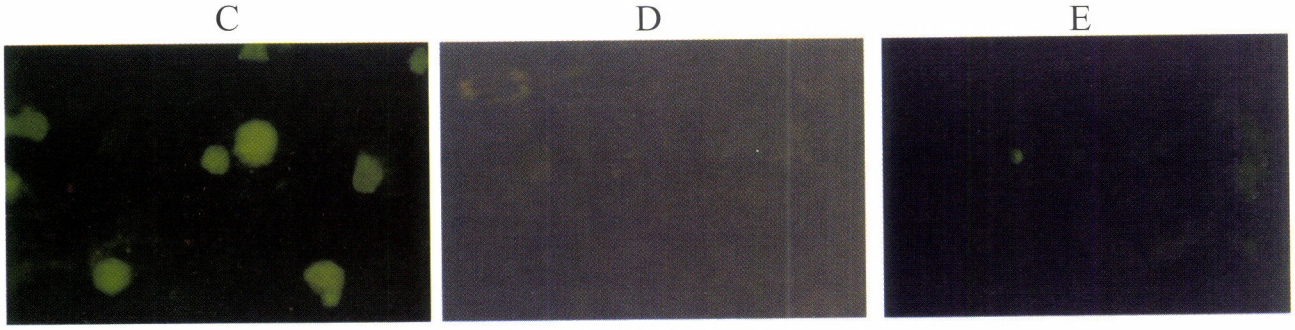

Fig. 6
Immunofluorescence staining of ICP4-transfected, reticuloendotheliosis virus-transformed lymphoblastoid cell lines
Cells were transfected with vectors with full-length (C) and 3.0 kbp ICP4 (D) and vector without ICP4 insert (E).

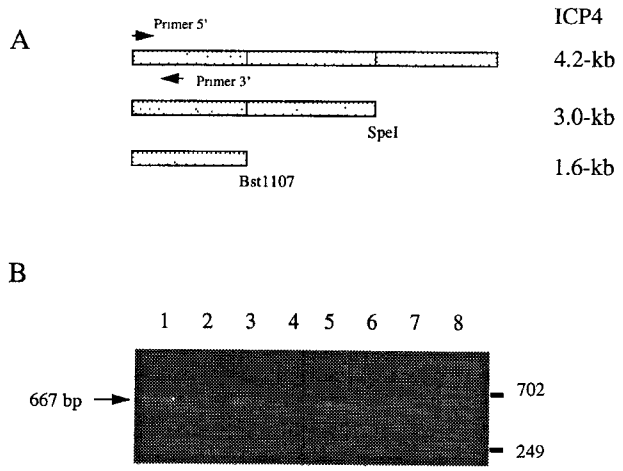

Fig. 5
ICP4 expression in ICP4-transfected, reticuloendotheliosis virus-transformed lymphoblastoid cell lines
(A) Cells were transfected with full-length and truncated ICP4 genes. The arrows indicate the location of the primers. (B) Detection of ICP4 transcripts in the transfected cells by RT-PCR and agarose gel electrophoresis. RT-PCR products obtained from cells transfected with vectors with full-length ICP4 (lane 1), 3.0 kbp ICP4 (lane 2) and 1.6 kbp ICP4 (lane 5) and vector without ICP4 insert (lane 7), respectively, treated or untreated (lanes 2,4,6 and 8, respectively) with RT prior to PCR.

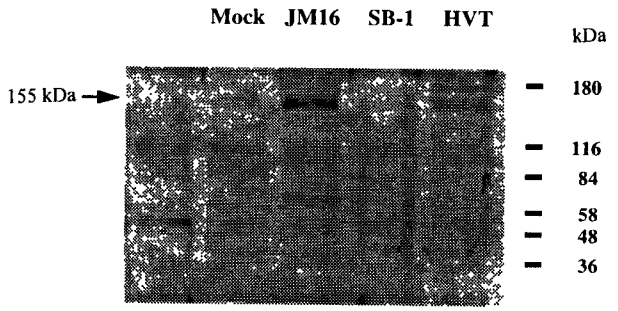

Fig. 7
ICP4 formation in CEFs infected with JM-16 and SB1 strains of MDV and HVT as determined by Western blot analysis
ICP4 was detected by MAb 5H8.

region downstream from the predicted polyA signal were able to detect ICP4 transcripts by Northern blot analysis.

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