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Recombinant Fowlpox Viruses Expressing the Glycoprotein B Homolog and the pp38 Gene of Marek's Disease Virus

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Two Marek's disease virus (MDV) genes, one homologous to the glycoprotein B gene of herpes simplex virus and encoding the B antigen complex and the other encoding a 38-kDa phosphorylated protein (pp38), were inserted into the fowlpox virus (FPV) genome under the control of poxvirus promoters. Randomly selected nonessential regions of FPV were used for insertion, and the vaccinia virus 7.5 kDa polypeptide gene promoter or a poxvirus synthetic promoter was used for expression of MDV genes. Gene expression in cells infected with these recombinants was highly influenced by the promoter (the synthetic promoter being more effective) but was only slightly influenced by the insertion site and by the transcription direction of the insert relative to the direction of the flanking FPV sequences. Cells infected with an FPV recombinant expressing the MDV gB gene reacted positively with a monoclonal antibody specific to this glycoprotein in an immunofluorescence assay. Immunoprecipitation of infected cell lysates showed three glycoproteins identical to those associated with the B antigen complex of MDV (100, 60, and 49 kDa). Cells infected with a recombinant expressing the pp38 gene reacted positively with an anti-pp38 monoclonal antibody in an immunofluorescence assay. The generated protein was phosphorylated and had a molecular weight similar to that of the native pp38 protein. Sera from chickens immunized with an FPV recombinant expressing the MDV glycoprotein B gene reacted with MDV-infected cells.

Marek's disease virus (MDV) is the causative agent of a highly contagious lymphoma of chickens. On the basis of its biological activities, it is classified as a gammaherpesvirus (22), but recent studies on the structure of its genome have indicated that it shares considerable structural similarities with alphaherpesviruses (3, 23). Homologs of the herpes simplex virus genes coding for glycoproteins B, C, D, and I (gB, gC, gD, and gI) and the gene coding for a 38-kDa phosphorylated protein unique to MDV (pp38) have been cloned and sequenced (6, 7, 23, 24).

Fowlpox virus (FPV) has a double-stranded DNA genome about 300 kb long (5) and has a host range limited to avian species (18). A number of nonessential regions of FPV has been identified and used for insertion of foreign DNA (2, 19). We have previously identified several randomly selected regions of FPV DNA and successfully used these regions as insertion sites for foreign DNA (33). In this study, we have used two of these regions for insertion of MDV genes.

The pp38 gene of MDV was cloned by screening an MDV λgt11 genomic library with a specific monoclonal antibody (8). Ross et al. (24) reported the cloning and sequencing of the MDV homolog of HSV gB. They synthesized peptides based on the deduced amino acid sequence of MDV gB and showed that the serum against the synthetic peptides reacted weakly with three proteins (110, 64, and 48 kDa).

We have constructed recombinant FPVs which express either the MDV pp38 gene or the gB gene. We detected a 38-kDa phosphorylated protein and three glycoproteins (gp100, gp60, and gp49) in cells infected with the recombinant FPVs containing these genes. These results suggest that the MDV gB encodes the B antigen complex, and posttranslational modification in recombinant FPV infected cells was similar to that in MDV-infected cells. We observed protective immunity against MDV with the recombinant expressing the MDV gB gene, which is discussed in another report (17).

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MATERIALS AND METHODS

Cells and viruses. The preparation, propagation, and infection of chicken embryo fibroblast (CEF) cell cultures with MDV and FPV have been described elsewhere (16, 30). A large-plaque variant (16) of a vaccine isolate of FPV was used for generation of recombinants.

DNA manipulation and sequencing. Cloning, restriction enzyme digestion, and other DNA manipulations were performed essentially in a conventional manner (14). For sequencing, DNA fragments were subcloned into a pUC18 vector and were treated with exonuclease III followed by mung bean nuclease to make sets of deletion mutants when necessary. The DNA sequence was determined by the dideoxy-chain termination method of Sanger et al. (26), using Sequenase kits (U.S. Biochemical, Cleveland, Ohio).

Construction of insertion vector pNZ1729R. The insertion vector pNZ1729R was used to transfer the pp38 and gB genes of MDV into the FPV genome. This vector was derived through multiple molecular manipulation of a cloned nonessential region of FPV DNA (33), including insertion of the *lacZ* gene as a reporter gene and creation of a synthetic poxvirus promoter with a multiple cloning site for insertion of foreign genes into this region (Fig. 1). A 3.0-kb *Hpa*I-*Spe*I fragment from a 7.3-kb *Eco*RI fragment (cloned in pNZ133) of pigeonpox virus (PPV) strain NP (19) was cloned into pUC18. After removal of remaining restriction sites between the cloned DNA and the plasmid vector, the multiple cloning site (*Hin*dIII-*Eco*RI; 52 bp) from pUC18 was inserted between two *Eco*RV sites within the cloned PPV DNA to give insertion vector pNZ133SR. A *lacZ* gene cassette was in-

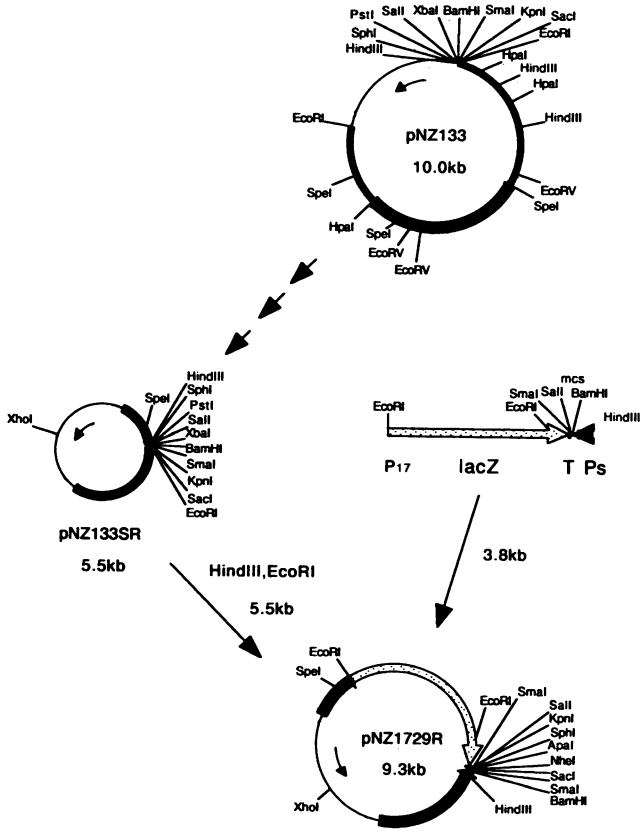

FIG. 1. Flowchart for the generation of insertion vector pNZ1729R (see Materials and Methods). Notation: bold lines, PPV DNA; stippled arrow, *lacZ* gene of *E. coli* with a PPV intrinsic promoter (P17) (arrow indicates the direction of transcription); small arrows, synthetic promoter (Ps); black bar, bidirectional poxvirus early transcription termination signal (T); mcs, multiple cloning site (*Sal*I, *Kpn*I, *Sph*I, *Apa*I, *Nhe*I, *Sac*I, *Sma*I, and *Bam*HI).

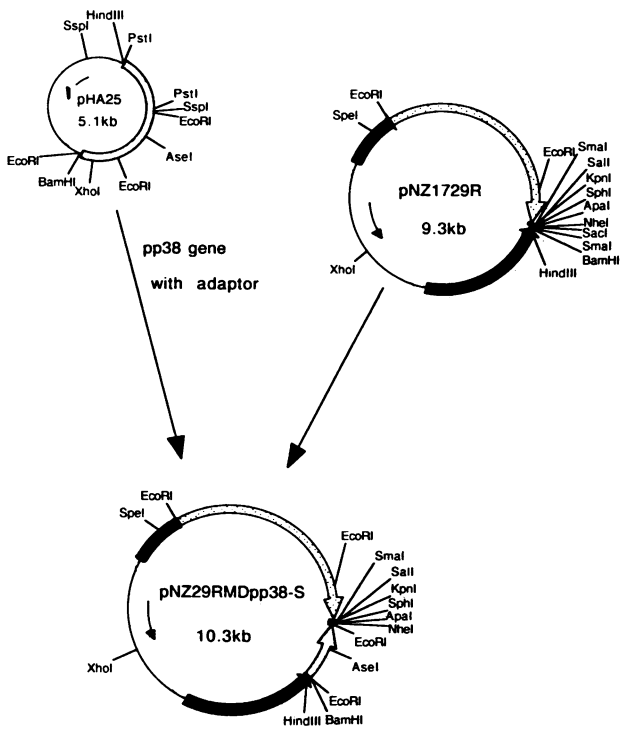

FIG. 2. Flowchart for the generation of transfer vector pNZ29RMDpp38-S for the MDV pp38 gene (see Materials and Methods). Notation: bold lines, PPV DNA; stippled arrow, *lacZ* gene of *E. coli* with a PPV intrinsic promoter; gray line, MDV DNA (gray line with arrow represents the pp38 gene); small arrows, synthetic promoter; black bar, bidirectional poxvirus early promoter transcription termination signal.

serted into this multiple cloning site. The gene cassette consists of three parts: (i) the *lacZ* gene of *Escherichia coli* (27) under the control of a PPV intrinsic promoter (5′-GTTGAAAAAATAATATA-3′ [19a]), (ii) a bidirectional early transcription termination signal (5′-ATTTTTATAAA AAT-3′ [34]) followed by a multiple cloning site

(5′-GTCGACGGTACCGCATGCGGGCCCGCTAGCGAGCTCGCCCGGGGATCC-3′)
*Sal*I *Kpn*I *Sph*I *Apa*I *Nhe*I *Sac*I *Sma*I *Bam*HI

and (iii) a synthetic promoter (Ps) (in opposite direction [5′-AAGCTTTTTTTTTTTTTTTTTTTTGGCATATAAAT AATAAATACAATAATTAATTACGCGTAAAAATTGA AAAACTATTCTAATTTATTGCACTCGGATCC-3′] which was optimized for early and late gene expression in vaccinia virus [9, 10]).

Construction of transfer vector pNZ29RMDpp38-S. The pp38 gene from plasmid pHA25 (7) was cloned into insertion vector pNZ1729R with a synthetic adaptor as shown in Fig. 2. Plasmid pHA25 was cleaved with *Eco*RI, ligated with a synthetic adaptor (5′-AATTCCATGATTGGATCCAAT-CATGG-3′), and digested with *Bam*HI and *Ase*I to obtain a 575-bp fragment. The plasmid was also digested with *Ase*I and *Ssp*I to recover a 450-bp fragment. *Bam*HI- and *Sma*I-digested plasmid pLEL95, which has an 137-bp *Hin*dIII-*Sal*I fragment of pNZ1729R in pUC18, was ligated with the 575-bp *Bam*HI-*Ase*I fragment and the 450-bp *Ase*I-*Ssp*I fragment. The entire pp38 gene was recovered as a 1.05-kb *Bam*HI-*Sal*I fragment and then inserted into pNZ1729R.

Construction of transfer vector pNZ29RMDgB-S. A 3.9-kb *Sal*I-*Eco*RI fragment containing a 2.8-kb *Bam*HI-*Sal*I subfragment of I_3 and a 1.1-kb *Bam*HI-*Eco*RI subfragment of K_3 from a *Bam*HI MDV strain GA genomic library (11) cloned in pWE15 was kindly provided by J. D. Reilly and was recloned in pUC18. The nucleotide sequence of the cloned fragment was determined by analyzing a set of deletion mutants by the dideoxy-chain termination method. One of these deletion mutants, pUCgBdB13, which contained the entire coding region of the gB gene, was chosen for insertion.

Plasmid pLELR was derived from pNZ1037 (19) by adding a synthetic adaptor,

5′-CGAATTCGTCGAC-3′
3′-TCGAGCTTAAGCAGCTGTTAA-5′,

to make a *Sal*I site next to an *Eco*RI site. It was digested with *Sma*I and *Eco*RI and was ligated with a 1.9-kb *Hin*dIII (Klenow-blunt)-*Bam*HI fragment and a 1.1-kb *Bam*HI-*Eco*RI fragment, both from pUCgBdB13. By using site-specific mutagenesis (31), about 250 bp of the 5′ flanking region of gB were deleted and a potential poxvirus early transcription termination signal in the gB gene in pUCgBP7.5 (TTTTTTT; nucleotides 26 to 32) was changed to TATTTTT. The oligonucleotide for site-specific mutagenesis of P7.5-gB was a 34-mer (5′-ACTCAATCAATAGCA ATCATGCACTATTTTAGGC-3′), and the oligonucleotide

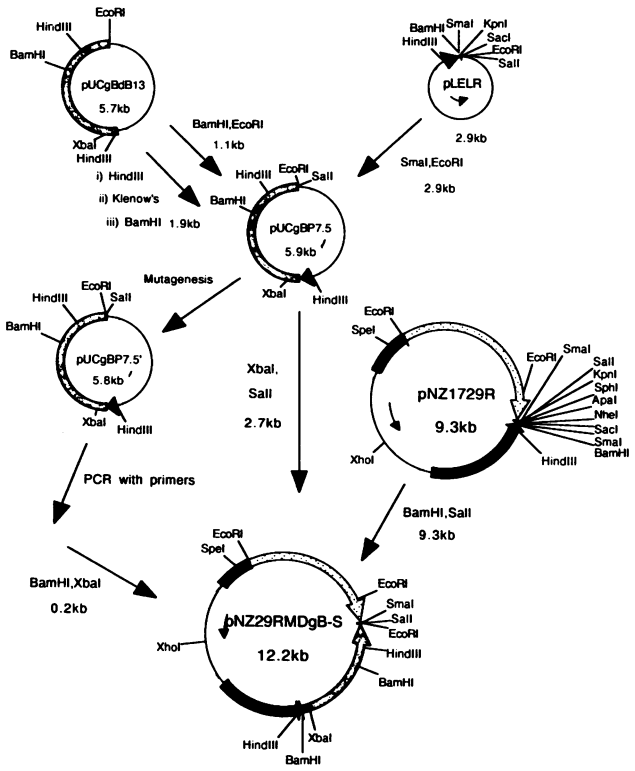

FIG. 3. Flowchart for the generation of transfer vector pNZ29RMDgB-S for the MDV gB gene (see Materials and Methods). Notation: bold lines, PPV DNA; stippled arrow, *lacZ* gene; gray lines, MDV DNA; gray line with arrow, gB gene of MDV; small arrows, synthetic promoter; black bar, bidirectional poxvirus early transcription termination signal.

for site specific mutagenesis of TTTTTTT was a 26-mer (5′-GCGGAATTGCATATTTTTCCTTATAG-3′).

To create a new *Bam*HI site in front of the translation initiation codon (ATG) of the gB gene for connecting the gB gene to a synthetic promoter, the polymerase chain reaction (25) was performed with synthetic oligonucleotides 5′-GG GATCCAATCATGCACTATTTTAGG-3′ (26-mer) and 5′-CGCGGCGGTTCTAGAC-3′ (16-mer). A *Bam*HI-*Xba*I fragment of about 200 bp from the polymerase chain reaction product was ligated with a 2.7-kb *Xba*I-*Sal*I fragment of gB and *Bam*HI-*Sal*I-digested vector pNZ1729R to make transfer vector pNZ29RMDgB-S (Fig. 3).

Construction of other transfer vectors. To compare the promoter activities between the vaccinia virus 7.5-kDa polypeptide promoter (P7.5) (32) and our synthetic promoter (Ps), we constructed transfer vectors pNZ29RMDpp38 and pNZ29RMDgB, which have P7.5 instead of Ps for antigen gene expression. We also constructed three different constructs based on the pp38 gene under control of the P7.5 promoter. One of these, named pNZ29LMDpp38, had the same structure as pNZ29RMDpp38 but with the inserted DNA fragment (the *lacZ* and pp38 genes with the promoters) in the opposite orientation. Two other transfer vectors, pNZ25RMDpp38 and pNZ25LMDpp38, were based on another insertion site (33) with the same inserts in two different orientations.

Generation and purification of recombinant FPVs. Procedures for transfection of FPV-infected cells with the transfer vector DNA by electroporation and generation of recombinants were described previously (19). Approximately 3×10^7 CEF cells previously infected with FPV at a multiplicity of infection of 0.1 were transfected with 10 μg of transfer vector. After 3 days of incubation, progeny FPV was assayed for expression of *lacZ* in the presence of Bluo-gal (600 μg/ml) in the agar overlay. Blue plaques were removed from agar and were clone purified until all FPV plaques were blue. This process usually took three to four passages.

Immunoprecipitation. SDS-PAGE, and Western immunoblot analysis. Immunoprecipitation of infected cell lysate was carried out as described previously (28). Secondary CEF cultures infected with either parental or recombinant FPV at a multiplicity of infection of 15 were incubated at 37°C for 4 h. The medium was then replaced with 1 ml of fresh methionine-free medium, and the cells were incubated for 1 h. About 40 μCi of [^{35}S]methionine (NEN, Wilmington, Del.) was added, and the cultures were incubated for 12 h. Similar cultures were incubated in phosphate-free medium containing 100 μCi of $^{32}P_i$ (NEN) to determine the phosphorylation of pp38 gene products. All other conditions were as described above. Cells were washed twice in phosphate-buffered saline (PBS), scraped, and transferred to a 15-ml Falcon tube. Cells were centrifuged, resuspended in lysis buffer (150 mM NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% sodium dodecyl sulfate [SDS], 10 mM Tris-HCl [pH 7.5]), and incubated at room temperature for 30 min. One-half volume of 10% (vol/vol) *Staphylococcus aureus* Cowan 1 was added to the cell lysate, which was then incubated for 30 min on ice. The lysate was then centrifuged, and the supernatant was collected. About 3 μl of monoclonal antibody H19 against pp38 or IAN86 against MDV gB (28) was added to 100 μl of lysate, which was then incubated for 30 min on ice. An equal volume of 10% (vol/vol) *S. aureus* Cowan 1 was added, and the lysate was incubated on ice for 30 min. Immunoprecipitates were then washed, suspended in sample buffer, and boiled. After centrifugation, the supernatant was analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) (13).

For Western blot analysis, lysates of infected cells were boiled at 100°C in sample buffer for 10 min and were loaded for SDS-PAGE as described elsewhere (13). After electrophoresis, the gel was soaked in transfer buffer (48 mM Tris, 39 mM glycine, 0.037% SDS, 20% methanol [pH 8.3]). Proteins were then blotted onto a nitrocellulose membrane by using an electrotransfer apparatus (Bio-Rad, Richmond, Calif.). After treatment with blocking buffer (5% skim milk–0.01% antifoam A–0.02% sodium azide in PBS), the membrane was incubated with mouse anti-pp38 monoclonal antibody H19 or anti-MDV gB monoclonal antibody IAN86 (28). The membrane was washed with PBS several times and then incubated with horseradish peroxidase-conjugated goat anti-mouse immunoglobulin. After being washed again with PBS several times, the membrane was incubated with substrate solution (6 mg of diaminobenzidine tetrahydrochloride in 9 ml of 10 mM Tris-HCl [pH 7.6], 1 ml of 0.3% $NiCl_2$, and 10 μl of 30% hydrogen peroxide) for 2 min.

Immunofluorescence. Coverslip cultures of CEF cells infected with parental FPV, recombinant FPVs, or MDV were washed in PBS and fixed with acetone. Coverslip cultures were then incubated with either monoclonal antibodies or anti-MDV chicken serum for 30 min at room temperature and washed extensively in PBS. Bound antibodies were detected with fluorescein-conjugated anti-mouse or anti-chicken immunoglobulin G.

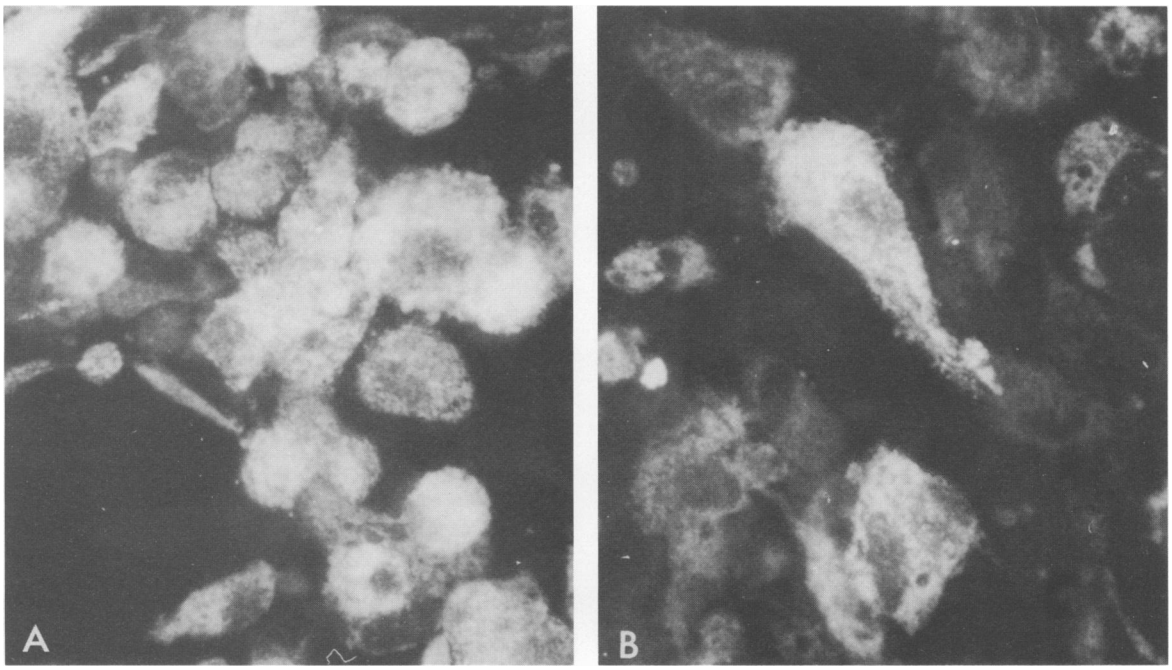

FIG. 4. Immunofluorescence staining of pp38 and gB produced in CEF cells infected with recombinant FPVs. Cells were infected with f29RMDpp38-S (A) or f29RMDgB-S (B). After several days of incubation, when plaques became visible, the cells were fixed with acetone and stained by indirect immunofluorescence.

RESULTS

Construction of recombinant FPVs; the pp38 gene of MDV. As shown in Fig. 2, the pp38 gene of MDV was cloned into insertion vector pNZ1729R with a synthetic adaptor to yield transfer vector pNZ29RMDpp38-S. We also constructed four other transfer vectors, pNZ29RMDpp38, pNZ29LMD pp38, pNZ25RMDpp38, and pNZ25LMDpp38, all having the vaccinia virus promoter (P7.5) instead of the synthetic promoter (Ps) (data not shown).

These transfer vectors were then used to transfect FPV-infected CEF cells. Purification of recombinant plaques was achieved by color selection as described in Materials and Methods. Purified recombinant virus from transfer vector pNZ29RMDpp38-S was named f29RMDpp38-S. Other recombinants were named similarly. These five recombinant viruses were very stable during several blind passages and showed the expected DNA fragment patterns in Southern blot hybridization analyses as predicted from double-crossover recombination events (data not shown).

Expression of the pp38 gene of MDV in recombinant FPV-infected cells. Figure 4A shows the immunofluorescent staining with an anti-pp38 monoclonal antibody of CEF cells infected with recombinant FPV f29RMDpp38-S. The pp38 gene products were observed only in the cytoplasm of the cells infected with the recombinants, and the intensity of the immunofluorescence with f29RMDpp38-S was stronger than that with f29RMDpp38.

Western blot analysis was conducted on extracts of uninfected cells and cells infected with MDV, parental FPV, and recombinant FPVs with the pp38 gene inserted in two different sites and in two different orientations and driven by two different promoters (Fig. 5). Cells infected with MDV contained a 38 kDa and a 41-kDa protein (lane 8). All FPV recombinants (lanes 3 to 7) contained only the 38-kDa protein, not the 41-kDa protein. Cells infected with either of two recombinants with the pp38 gene inserted in one position (lanes 3 and 4) or with two recombinants with the insert in the second position (lanes 5 and 6) did not differ significantly in the amount of pp38 expressed. Also, the orientation of the insert relative to the flanking FPV sequences (lane 3 compared with lane 4 and lane 5 compared with lane 6) did not significantly influence the expression of pp38. However, cells infected with the recombinant in which the pp38 gene was driven by the synthetic promoter (lane 7) expressed

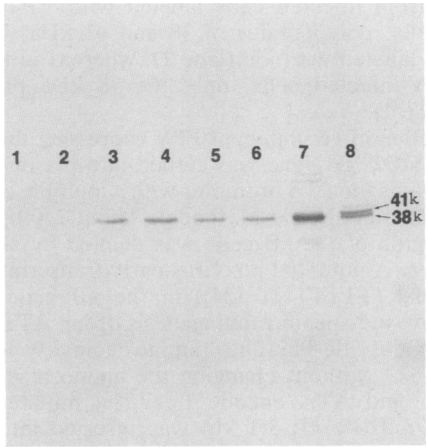

FIG. 5. Comparison by Western blot analysis of pp38 gene expression in cells infected with different recombinant FPVs, using monoclonal antibody H19. Lanes: 1, CEF cells; 2, parental FPV-infected cells; 3 to 8, cells infected with f25LMDpp38, f25RMD pp38, f29LMDpp38, f29RMDpp38 (all four driven by P7.5 promoter), f29RMDpp38-S (driven by a poxvirus synthetic promoter), and MDV GA, respectively.

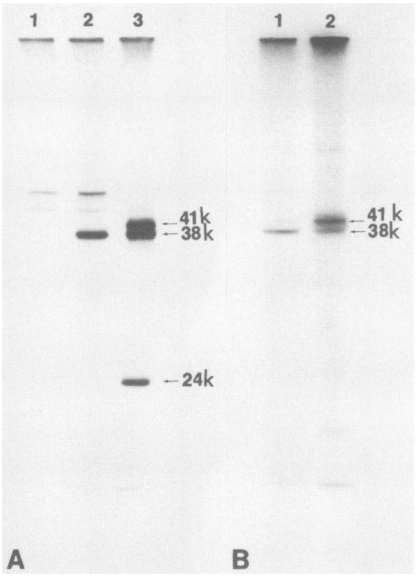

FIG. 6. Identification of the pp38 gene product by immunoprecipitation. Immunoprecipitation was performed with monoclonal antibody H19. (A) [^{35}S] methionine labeled. Lanes: 1, parental FPV; 2, f29RMDpp38-S; 3, MDV GA. (B) $^{32}P_i$ labeled. Lanes: 1, f29RMDpp38-S; 2, MDV GA.

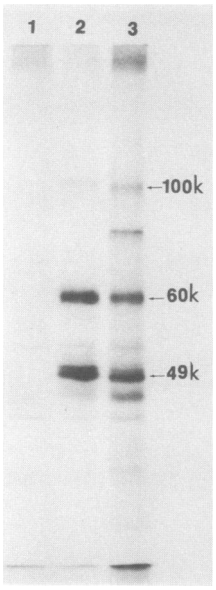

FIG. 7. Identification of the gB gene product by immunoprecipitation. Immunoprecipitation was performed with monoclonal antibody IAN86. Lanes: 1, parental FPV; 2, f29RMDgB-S; 3, MDV GA.

about four-times-higher levels of the protein, as estimated by densitometer measurements, compared with genes driven by the P7.5 promoter (lanes 3 to 6). Expression of the pp38 gene in CEF cells infected with MDV or recombinant FPV was also examined in an immunoprecipitation assay. Figure 6A shows the immunoprecipitation of [^{35}S]methionine-labeled proteins of cells infected with parental FPV (lane 1), recombinant FPV expressing the pp38 gene (lane 2), and MDV (lane 3). Three proteins with molecular sizes of 24, 38, and 41 kDa were detected in MDV-infected cells, whereas only a single 38-kDa polypeptide was detected in recombinant FPV-infected cells. Cells infected with parental FPV lacked all three polypeptides. Figure 6B shows similar immunoprecipitation from infected cells labeled with $^{32}P_i$. In MDV-infected cells, polypeptides of 38 and 41 kDa (but not 24 kDa) were labeled with ^{32}P (lane 1), whereas in the recombinant FPV-infected cells, only the 38 kDa protein was labeled (lane 1).

Construction of recombinant FPV expressing the MDV gB gene. The MDV gB gene was cloned into plasmid pLELR, which contains the P7.5 promoter with a multiple cloning site in pUC18 (Materials and Methods). About 250 bp of the 5′ flanking region of the gB gene was deleted by site-specific mutagenesis. A potential poxvirus early transcription termination signal (TTTTTNT [34]) in the gB gene was also modified by site-specific mutagenesis from ATTTTTTTC, which encodes Ile-Phe-Phe (amino acids 9 to 11), to ATATTTTTC, without changing the amino acid sequence (both ATT and ATA encode Ile). The mutated P7.5-gB cassette (*Hin*dIII-*Sal*I; 3.1 kb) was inserted into *Hin*dIII-*Sal*I-digested pNZ1729R to make the transfer vector pNZ29RMDgB. Another transfer vector, pNZ29RMDgB-S, was also constructed as described in Materials and Methods.

Two recombinant FPVs, f29RMDgB and f29RMDgB-S, were generated from transfer vectors pNZ29RMDgB and pNZ29MDgB-S, respectively. The stability of these viruses was determined after several blind passages by examining the expression of β-galactosidase. The correct insertion of the *lacZ* and gB genes was confirmed by Southern blot hybridization analysis (data not shown).

Expression of the MDV gB gene in cells infected with recombinant FPV. Figure 4B shows the immunofluorescence of CEF cells infected with recombinant FPV f29RMDgB-S. Positive stainings were observed only in the cytoplasm of cells infected with recombinants. Similar to cells infected with the pp38 gene recombinants, the cells infected with the synthetic promoter-driven recombinant expressed higher levels of the MDV gB.

Western blot analysis of proteins from recombinant FPV-infected cells did not reveal the expected glycoprotein bands associated with gB when lysates were boiled in sample buffer. However, we detected a high-molecular-weight band with an R_f value similar to that in MDV-infected cell lysates solubilized with sample buffer at room temperature instead of 100°C. To show clearly the three species of glycoproteins previously shown to be associated with MDV B antigen complex, we examined the expression of the gB gene by immunoprecipitation. Figure 7 shows the result of immunoprecipitation with a monoclonal antibody (IAN86) specific to the MDV B antigen complex. Three identical bands of 100, 60, and 49 kDa were observed in extracts of cells infected with f29RMDgB-S (lane 2) and MDV (lane 3). These polypeptides were also shown to be glycosylated (data not shown). Similar glycoproteins were identified with the same monoclonal antibody in the MDV B antigen complex and were referred to as gp100, gp60, and gp49 (29). The latter two are believed to be the cleavage products of gp100.

Immunological responses of chickens against recombinant FPVs. Three groups of five 3-week-old specific-pathogen-free line 0 chickens were injected intramuscularly with 10^6 infectious doses of f29RMDpp38-S, f29RMDgB-S, or parental FPV. The chickens were boosted two times (2 and 4 weeks after the first inoculation). After another 2 weeks, the birds were bled and sera were collected. Sera from chickens immunized with f29RMDgB-S reacted positively with MDV-infected cells by immunofluorescence (data not shown). Sera

from chickens immunized with f29RMDpp38-S or the parental FPV failed to stain the MDV antigens in a similar assay.

DISCUSSION

In this report, we describe the construction of a number of recombinant FPVs expressing the MDV gB and pp38 genes. Two randomly selected nonessential regions of FPV were used for generation of these recombinants. Transfer vectors used were originally obtained from clones of PPV (19). These clones were successfully recombined with homologous sequences of FPV to generate stable recombinants. We have determined that the DNA sequences of PPV and FPV are highly conserved and that the restriction enzyme pattern of these two viruses are quite similar (data not shown). We used a weak promoter (P17) to drive expression of the *lacZ* reporter gene, because strong expression is not necessary and may cause adverse effects. We also used a bidirectional transcription termination signal between the *lacZ* gene and the antigen gene. This signal is utilized in vaccinia virus (34) and suggested (21) to function in the FPV system.

Insertion of MDV genes into either of the insertion sites used in these studies or in either orientation generated stable recombinants that expressed these genes. However, insertion in one of these sites (pNZ25) alters the phenotype of the recombinant virus and results in smaller plaques and lower titer of released virus in the medium (unpublished data). Recombinants generated as a result of insertion within the pNZ29 sequences remained stable without any phenotypic change in CEF cells. We have chosen this site as the most suitable site for insertion.

Of the two promoters that we used to drive the expression of MDV genes, the synthetic promoter, which was optimized for gene expression in vaccinia virus (9, 10), was much more effective than the vaccinia virus early-late P7.5 promoter. This is not surprising, because similarities in transcription signal between the two viruses have been documented (1, 21).

pp38 is an important protein because it is a putative transformation-related viral antigen (7) and is one of a few proteins expressed in lymphoid tumor cells (7, 15). Cui et al. (8) reported the cloning of the pp38 gene by immunoscreening of a λgt11 genomic library of MDV. They mapped the pp38 gene to the *Bam*HI H or *Eco*RI X fragment of the MDV genome and sequenced it (7). They showed that immunoprecipitation of lysates from MDV-infected cells with antiserum against a pp38-LacZ fusion protein identified not only a 38-kDa phosphorylated protein but also two glycoproteins of 60 and 49 kDa. They also found the fusion protein to cross-react with both anti-pp38 and anti-antigen B monoclonal antibodies.

In this study, we have identified three polypeptides in MDV-infected cell lysate that immunoprecipitated with an anti-pp38 monoclonal antibody. Of these three, the 41- and 38 kDa proteins were phosphorylated but the 24-kDa protein was not. In cells infected with recombinant FPV possessing the pp38 gene, only the 38-kDa protein, which was also phosphorylated, was identified. The 24-kDa protein was detected in immunoprecipitates (Fig. 6) but not in Western blots (Fig. 5) of MDV-infected cell lysates. Therefore, the protein is most likely not immunogenically related to pp38 but rather coimmunoprecipitated with pp38. On the other hand, the 41-kDa protein was detected both in Western blots (Fig. 5) and in immunoprecipitates (Fig. 6) from MDV-infected cell lysates and not in recombinant FPV-infected cell lysates. These data suggest that the 41-kDa phosphorylated protein is either an additionally phosphorylated product of pp38 or an immunogenically related protein encoded by another gene of MDV.

Although all pp38 recombinant FPVs expressed considerable amounts of pp38 in infected cells, sera from chickens immunized with f29RMDpp38-S failed to react with MDV antigens in immunofluorescence and virus neutralization assays (17). These data suggest that, in contrast to the three glycoproteins encoded by the MDV gB gene, pp38 expressed in recombinant FPV did not induce a good immunological response in the chickens.

An antigen referred to as the B antigen was originally identified in MDV-infected cells by Churchill et al. (4) and is believed to be of significance because it is involved in virus neutralization (12) and can elicit partial protection against Marek's disease (20). Sithole et al. (29) reported that the gene encoding the B antigen complex of MDV was localized to the viral genome's *Bam*HI D and H fragments, as determined by hybrid selection and cell-free translation. On the other hand, Ross et al. (24) reported cloning the gB gene of MDV strain RB1B by random sequencing of the viral genome and localized this gene to the *Bam*HI I_3 and K_3 fragments. We cloned and sequenced the gB gene of the GA strain of MDV and found that the nucleotide sequence for this gene is identical to that reported for the RB1B strain by Ross et al. (24).

Immunoprecipitation studies revealed that the CEF cells infected with recombinant FPVs harboring the MDV gB gene expressed three glycoproteins: gp100, gp60, and gp49. This is the first clear demonstration that the MDV gB gene codes for these three glycoproteins previously referred to as the B antigen complex. The latter two glycoproteins are believed (29) to be the cleavage products of gp100. Since the monoclonal antibody used in this study (IAN86) has been reported to neutralize MDV infectivity and was found to react with the glycoproteins produced in cells infected with f29RMDgB-S, this recombinant is a good candidate for a vaccine against Marek's disease. The ability of this recombinant to protect against Marek's disease is reported in the accompanying paper (17).

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