Assembly of Marek’s disease virus (MDV) capsids using recombinant baculoviruses expressing MDV capsid proteins

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The genes UL18, UL19, UL26, UL26.5, UL35 and UL38 of Marek’s disease virus 1 (MDV-1) strain RB1B, encoding the homologues of herpes simplex virus type 1 (HSV-1) capsid proteins VP23, VP5, VP21–VP24, preVP22a, VP26 and VP19C, were identified and sequenced. Recombinant baculoviruses were used to express the six capsid genes in insect cells. Coexpression of the six genes or of UL18, UL19, UL26.5 and UL38 in insect cells resulted in the formation of capsids with a large core. In addition, electron microscopy of thin sections clearly revealed the presence of large numbers of small spherical particles. Experimental coinfection demonstrated that these small particles were associated with production of the preVP22a protein.

Marek’s disease virus (MDV) is an alphaherpesvirus that causes contagious malignant T-cell lymphoma in chickens (Calnek, 2001). Three MDV serotypes can be distinguished on the basis of virulence in chickens: serotype 1 (MDV-1), corresponding to all MDV strains with oncogenic potential; serotype 2 (MDV2), corresponding to naturally occurring nononcogenic MDV strains, and serotype 3 (MDV3), corresponding to the nononcogenic herpesvirus of turkey. Like others members of this family, the virions of Marek’s disease-like viruses consist of four distinct components: an electron-dense core containing the viral DNA, an icosahedral capsid, an amorphous layer known as the tegument, and an envelope displaying the virus glycoprotein spikes on its surface. The complete coding sequences of several Marek’s disease viruses have recently been determined (Afonso et al., 2001; Lee et al., 2000; Tulman et al., 2000), but little is known about the protein composition of the capsid (Kato et al., 2001; Lee et al., 2000; Tulman et al., 2000). Previous studies on the prototype alphaherpesvirus herpes simplex virus type 1 (HSV-1) have shown that the herpesvirus capsid is built by assembling at least seven virus proteins (Gibson & Roizman, 1974; Heilmann et al., 1979; Newcomb et al., 1993; Vernon et al., 1981). In 1994, the production of intermediate HSV-1 capsids in insect cells infected with six recombinant baculoviruses producing the seven capsid proteins was reported (Tatman et al., 1994; Thomsen et al., 1994). However, to date, assembly of homologous recombinant capsid proteins has not been described for other herpesviruses. Hybrid capsids were obtained with HSV-1 scaffold protein substituted for the corresponding bovine herpesvirus (BHV) or varicella-zoster virus (VZV) scaffold proteins (Haanes et al., 1995; Preston et al., 1997). In this study, we expressed the genes of hypervirulent MDV-1 (strain RB1B) that are homologous to the HSV-1 genes UL18, UL19, UL26, UL26.5, UL35 and UL38 in insect cells, using a baculovirus system to determine their ability to assemble into capsids.

The hypervirulent strain of MDV-1, RB1B, was maintained as described previously (Djeraba et al., 2000). Virus particles were isolated from feather follicles obtained from chickens infected with MDV-1-RB1B. After extraction and purification, 4 μg RB1B viral DNA was digested overnight with 100 U of BamHI. A library of purified BamHI restriction fragments of the MDV-1-RB1B genome was made using the pBluescript vector (pBS). For the selected clones, we determined the nucleotide sequences at the extremities of the inserts and used FASTA and BLAST to look for similarities to published alphaherpesvirus sequences in databases. This enabled us to determine the location of the inserts in the MDV genome (Fig. 1). Twenty-three of the 29 BamHI fragments shown on the BamHI map of MDV-1-GA DNA were obtained from the library. The BamHI fragments containing the homologues to genes UL18, UL19, UL26, UL26.5, UL35 and UL38 from HSV-1 were sequenced fully (accession nos AF439268, AF439269, AF439270 and AF439271). We named the MDV-1-RB1B genes and proteins according to their homology with the HSV-1 capsid genes: UL18, UL19, UL26, UL26.5, UL35 and UL38, encoding proteins VP23, VP5, VP24–VP21, preVP22a, VP26 and VP19C, respectively. The MDV-1-RB1B UL18, UL19, UL26, UL26.5, UL35 and UL38 genes were 960 nt, 4182 nt, 1992 nt, 1038 nt, 396 nt and 1410 nt long, respectively. For VP24–VP21, by analogy with the corresponding HSV-1 protein, the release (R) site was located between amino acids Ala-234 and Ser-235 in the protease and the maturational (M) site was located between amino acids Ala-638 and Ser-639, at the C-terminal end of the assembly protein precursor.
Alignments of the nucleotide sequences of the capsid genes of strains MDV-1-RB1B, MDV-1-Md5 and MDV-1-GA showed that the MDV-1-GA and MDV-1-RB1B sequences (94.5% identical) are less similar than those of MDV-1-Md5 and MDV-1-RB1B (99-99% identical). The sequences of the UL18, UL35 and UL38 genes of MDV-1-Md5 and MDV-1-RB1B are absolutely identical, and only one mutation, in each of UL19 and UL26, was detected between the two strains. A larger number of point mutations are evident between the MDV-1-RB1B and MDV-1-GA capsid genes, but the most important variations correspond to insertions/deletions: (i) one codon (286–288) deleted in the UL35-GA gene; (ii) two successive deletions (19–40 and 914–931) in the UL19-GA gene leading to seven and five amino acid changes, respectively, in the VP5-GA protein; (iii) one insertion (218) and one deletion (284), leading to changes in 22 amino acids in the VP23-GA protein; (iv) one deletion (1587) associated with two insertions (1737 and 1802), leading to a change in peptide sequence before amino acid 530, resulting in the truncation of proteins VP21-GA and preVP22a-GA, and elimination of the predicted M site. These substantial differences could be investigated by resequencing the GA capsid genes, given that sequencing errors could be responsible.

Recombinant baculoviruses were produced with the Bac-to-Bac baculovirus expression system (Gibco-BRL). The various genes encoding capsid proteins were generated by PCR from plasmids containing the genes corresponding to UL18, UL26, UL26.5 and UL35 or with DNA extracted from strain MDV-1-RB1B for UL19 and UL38. The primers used were as follows: UL19 (tctaga gcggccgcTATCAGTTACAATCTCAGC, tctaga gcggccgcATG GCCGGATGCCATTGTC); UL18 (tctaga gcggccgcCAACATACTAGCATGAATATGA, tctaga gcggccgcATG AGTACTTCCAACGGCACG); UL26 (at gcggccgcGCTCCCTGTATTATTGATGC, at gcggccgcATG AACACTCAATCTTCTCG); UL26.5 (at gcggccgcGCTCCCTGTATTATTGATGC, at gcggccgcATG AACTCTAATCCTTCTCG); UL35 (gcggccgcTTATGACGTCGATATATCATCATCT, gcggccgcATG TCTCCTGTATTTGTC); UL38 (gcggccgcTCA TTAATAACATTCGATCCATGTACC, gcggccgcATGAACACTCAATCCTTCTCG); Not I sites are underlined, and the initiation codons are shown in bold. The PCR fragments were separated by electrophoresis, purified from an agarose gel and inserted into the T-tailed vector pGEM-T (Promega). We checked the inserts by sequencing, and inserted the six genes into the Not I site of the pFastBac1 shuttle vector. Recombinant baculoviruses BacUL18, BacUL19, BacUL26, BacUL26.5, BacUL35 and BacUL38 producing VP23, VP5, VP24–VP21, preVP22a, VP26 and VP19C proteins, respectively, were generated using the Bac-to-Bac system according to the vector manufacturer’s instructions. With the exception of BacUL26, all the recombinant...
baculoviruses produced the desired proteins specifically and in abundance. These proteins had estimated molecular masses (eMMs) on SDS-PAGE similar to their predicted molecular masses (pMM) (Fig. 2A, B). Thus, BacUL18 produced a protein with an eMM of 33 kDa corresponding to VP23 (pMM = 35 kDa), BacUL19 produced a protein with an eMM of 155 kDa corresponding to VP5 (pMM = 155 kDa), BacUL26.5 produced a protein with an eMM of 38 kDa corresponding to preVP22a (pMM = 38 kDa), BacUL35 produced a protein with an eMM of 14 kDa corresponding to VP26 (pMM = 14-5 kDa) and BacUL38 produced a protein with an eMM of 53 kDa corresponding to VP19C (pMM = 52-5 kDa). For BacUL26, we detected only a faint band with an eMM of 26 kDa that may be specific to the VP24 protein (Fig. 2A). Immunoblot analysis with monoclonal antibodies specific for VP21 and VP24 confirm that BacUL26 expressed these proteins (Fig. 2C, D). It should be noted that the loss of a band corresponding to the fusion protein VP21–VP24 indicates a complete cleavage of this fusion protein at the R site into the two products VP21 and VP24 at 64 h post-infection. However, the precise site of cleavage was not checked.

To determine whether the MDV-1-RB1B capsid proteins expressed by the recombinant baculoviruses could self-assemble to form MDV-1 capsids, monolayers of SJ21 cells were infected with BacUL18, BacUL19, BacUL26, BacUL26.5, BacUL35 and BacUL38 in various combinations, using an m.o.i. of 5 for each virus. The cells were cultured and harvested 64 h after infection. Thin sections were prepared for electron microscopy, as described by Preston et al. (1983), and observed using a Philips MC10 electron microscope. When monolayers of SJ21 cells were infected with all the recombinant baculoviruses (BacUL18, BacUL19, BacUL26, BacUL26.5, BacUL35 and BacUL38) or four of the six recombinant baculoviruses (BacUL18, BacUL19, BacUL26.5 and BacUL38), identical spherical structures were observed that were not present in cells infected with the parental AcRP6-SC virus (Fig. 3A–C). In both assays, the particles with a diameter of about 100 nm were produced in the nuclei. They contained a large internal core structure about 60 nm in diameter, typical of immature capsids formed in MDV-infected cells (Ahmed & Schidlovsky, 1968). These particles were similar in appearance to the ‘large-core’ capsids observed in one experiment in which cells were infected by one HIV-1 mutant that has a lesion in the protease (Rixon & McNab, 1999). However, unlike HSV-1 capsids assembled using recombinant baculoviruses, all of the capsids observed had a ‘large-core’ appearance whether BacUL26 was present (Fig. 3B) or not (Fig. 3C). Further study of thin sections of infected cells showed that there were 20 times more capsids per cell if BacUL26 and BacUL35 were omitted from the mixture used for coinfection than if all six recombinant baculoviruses were used (data not shown). In HSV-1, the VP26 protein was found to play an important but non-essential role in capsid formation in a study by Ahmed & Schidlovsky, 1968)

Fig. 2. (A, B) Production of MDV-1-RB1B capsid proteins in cells infected with recombinant baculoviruses. Proteins synthesized in cells infected with parental virus AcRP6-SC (AcRP6) or cells infected with recombinant baculovirus BacUL38, BacUL26.5, BacUL26, BacUL18, BacUL19 and BacUL35 were separated by electrophoresis in 7-5 % (A), or 16-5 % (B) polyacrylamide gels containing SDS, and the proteins were detected by Coomassie blue staining. The MDV-1-RB1B capsid proteins produced by each recombinant baculovirus are indicated by an asterisk. (C, D) Immunoblot analysis of proteins expressed by recombinant baculoviruses BacUL26 and BacUL26.5. Protein blots of BacUL26- or BacUL26.5-infected cell proteins were separated by 10 % SDS-PAGE gel and probed with (C) the supernatant of specific monoclonal antibody Hb5 against the scaffold proteins (dilution 1:2 in PBS) or with (D) the supernatant of specific monoclonal antibody 8b6 against the protease (dilution 1:4 in PBS). Visualization was performed using an ECL detection system (Amersham) as described by the manufacturer.
Fig. 3. Electron micrographs of thin sections of Sf21 cells infected with recombinant baculoviruses or with parental virus AcRP6-SC. Sf21 cells, infected with various combinations of recombinant baculoviruses (each at 5 p.f.u. per cell), were harvested at 64 h. After fixation, thin sections were prepared for electron microscopy as described by Preston et al. (1983). Panels (A) to (G) show electron micrographs of thin section of cells infected with the following viruses: A) AcRP6-SC, B) all six recombinant baculoviruses, C) BacUL18, BacUL19, BacUL26.5 and BacUL38, D) BacUL26.5, E) BacUL26.5 and BacUL19, F) BacUL26.5 and BacUL18 and G) BacUL26.5 and BacUL38. Capsids (Ca), small particles (SPs) and baculovirus capsids (Bc) are indicated by arrows. Bars, 100 nm.
Thomsen et al. (1994). However, other studies reported no clear effect on the formation or appearance of HSV-1 capsids (Newcomb et al., 1996; Tatman et al., 1994). It seems unlikely that a negative effect of the VP26 protein of MDV-1-RB1B could account for the reduction in capsid numbers, and more probably that the VP24–VP21 protein encoded by UL26 was responsible. In all expression assays, S21 monolayer cells infected with BacUL26 lysed 24 h before S21 monolayer cells infected with the other recombinant baculoviruses. This early cytopathic effect may be correlated with the particularly low levels of VP24–VP21 protein production by BacUL26. Similarly low levels of VP24–VP21 protein production in the baculovirus system have also been described for another alphaherpesvirus, VZV, with this protein detectable only in [35S]methionine-labelled virus-infected cell extracts (Preston et al., 1997). The protease may also prevent self-assembly of the capsid by early cleavage at the M site in the scaffold proteins. This cleavage would prevent the VP5–preVP22a or VP5–VP24–VP21 interaction (Desai & Person, 1996; Hong et al., 1996), thereby inhibiting the formation of the capsid (Kennard et al., 1995; Thomsen et al., 1995). Thus, during coinfection of S21 cells with all six recombinant baculoviruses, the ‘large-core’ capsids might only be produced in S21 cells that did not express the protease. Further studies are required to confirm the location of the M site and to evaluate the specificity of the MDV scaffold protein in capsid assembly.

In addition to capsids, small particles (about 60 nm in diameter) with an electron-lucent centre were present in the nucleus of several infected cells (Fig. 3B,C). We investigated the composition of these small particles by infecting S21 cells with BacUL18, BacUL19, BacUL26.5 and BacUL38 separately or with all possible pairwise combinations of these recombinant baculoviruses. We examined the cells infected with the various baculoviruses and found that large numbers of aggregates or areas of scaffold-like particles 60 nm in diameter were detected only if the cells were infected with BacUL26.5, either alone, or in combination with another baculovirus (Fig. 3 D–G).

In this study, we demonstrated the successful assembly of MDV-like capsids by expressing capsid genes in the baculovirus system. To date, the only other herpesvirus for which capsids have been obtained in a heterologous expression system is the alphaherpesvirus HSV-1 (Tatman et al., 1994; Thomsen et al., 1994). Coexpression of the UL18, UL19, UL26.5 and UL38 genes of MDV-1-RB1B, encoding VP23, VP5, preVP22a and VP19C, respectively, was found to be necessary and sufficient for capsid production.

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References


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