Expression of the Nucleocapsid Protein Gene of the Canine Distemper Virus

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ABSTRACT. We constructed a cDNA clone of canine distemper virus (CDV) encoding an entire nucleoprotein (NP) gene, by means of the reverse transcription-polymerase chain reaction (RT-PCR). The cloned NP gene was inserted into the eucaryotic expression vector, pRVSV. After transfection of the plasmid into Vero cells, we examined the expression of CDV-specific NP antigen by means of indirect immunofluorescence assay (IFA) and Western blotting, using various antibodies against NP of CDV and an antiserum against NP of measles virus. The CDV-NP specific antigen was detected in the nuclei of the cells transfected with pRV-ON, by means of IFA with antibodies specific to the NP. — KEY WORDS: CDV, expression, NP.


Canine distemper virus (CDV) belongs to genus Morbillivirus in the family Paramyxoviridae. In all negative-stranded RNA viruses including the paramyxoviruses, the genomic RNA is tightly bound to nucleoprotein (NP), the major structural protein, in the form of a helical nucleocapsid. For virus replication, the genomic RNA-nucleocapsid is required as a template and transcription proceeds in vitro in the presence of phosphoprotein and the large protein [3]. To further understand the mechanisms of viral protein-interaction in CDV replication, it is important to analyze the gene coding the NP, which plays an important role in nucleocapsid formation. Here, we cloned a cDNA of the CDV NP gene by reverse transcription-polymerase chain reaction (RT-PCR), and analyzed the 5'-terminal sequence to determine whether or not the cDNA encodes the entire open reading frame (ORF) of the NP gene. We inserted the cloned NP gene into a eucaryotic expression vector and expressed it in Vero cells to confirm whether or not the expressed protein has the same characteristics of the CDV NP. The expressed product was identified by means of Western blotting analysis using monoclonal antibodies (MAbs) against CDV NP and antiseras against measles virus (MV) NP and CDV. We also examined the cellular localization of NP by means of the indirect immunofluorescence assay (IFA). This is the first report to describe the expression of CDV NP in eucaryotic cells.

Vero cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 5% fetal calf serum and antibiotics. To prepare viral RNA, Vero cells were inoculated with the laboratory Onderstepoort strain of CDV. After adsorption for 60 min at 37 °C, the infected cells were incubated for 18 hr at 37 °C, then total RNA was extracted from the cells [2]. To obtain cDNA of CDV NP, RT-PCR proceeded as follows. Total RNA (5 µg) was reverse transcribed by reverse transcriptase (GIBCO BRL Life Technologies Inc, Gaithersburg, Md., U.S.A.) in the presence of the antisense primer, P2 (1570–1587 nt position of NP gene of the Onderstepoort strain) [7, 11]. The resulting cDNA was amplified in the same tube by means of PCR (Perkin-Elmer Cetus, Norwalk, Conn., U.S.A.) [8], in the presence of the above and random primers. Thereafter, the products were phosphorylated and ligated into a pUC118 cloning vector and designated as pUC-ON (Fig. 1). The DNA sequences were determined using the Sequenase Version 2.0 kit (United States Biochemical Co., Cleveland, Ohio, U.S.A.). The size and sequence of the NP gene determined in this study, were identical with those reported [7, 11].

We studied the expression of the cDNA clone using the

![Fig. 1. Schematic summary of the primers and the vectors used in this study. The sequence of 5' end of NP cDNA was identical except one nucleotide with those reported by Sidhu et al. [11] (5'-TAAAAATTTAGGGTCAATGATCCTACCCTTCTTAAAAGCCTCACACT-3'). A 1,639 bp PCR product was inserted into the pUC118 cloning vector and recloned into pRVS1 expression vector [9]. The constructed plasmids were designated pUC-ON and pRV-ON plasmids, respectively. To construct the pUC-ON plasmid, the fragment was blunt-ligated and ligated with the blunt SalI site of pUC118. After sequencing, the fragment was inserted into the BamHI and XbaI site of pRVS1 to construct pRV-ON.](image-url)
plasmid, pRVSV (excluding the neo gene of pRVSVneo) [9]. Plasmid pRVSV harbors the promoter sequence from the Rous sarcoma virus (RSV) and the poly A signal of SV40. To construct an NP expression vector, the entire NP gene of about 1.7 kbp from pUC-ON was ligated into pRVSV (Fig. 1). The constructed plasmid, pRV-ON, contained one protein-coding exon of the complete NP gene, located between the promoter sequence and the poly A signal. Vero cells cultured to near confluence were transfected with 10 µg of pRVSV or pRV-ON DNA per plate by means of calcium phosphate-DNA coprecipitation [1]. The cells were harvested 48 hr later. To determine the expression, IFA and Western blotting were performed using two antisera and two MAb that recognize epitopes of CDV NP [4]. The positive control was Vero cells infected with the Onderstepoort strain of CDV.

IFA showed that two MAb, c5 and f5 [4], positively reacted with Vero cells transfected with pRV-ON, and infected with CDV. The NP was detected in the nuclei of the cells transfected with pRV-ON, whereas it was abundant in the cytoplasm of cells infected with CDV (Fig. 2: f5, A to C; c5, D to F). Five MAb against CDV hemagglutinin and fusion proteins did not stain the transfected cells although they reacted with the cells infected with CDV as clearly as the MAb against NP (data not shown).

The lysates of transfected cells were Western blotted to estimate the molecular weight of the expressed NP. The proteins were resolved by electrophoresis on 10% sodium dodecysulfate polyacrylamide gels, then transferred to a Hybond-C extra membrane (Amersham, Buckinghamshire, UK). The membrane was then incubated at room temperature with MAb against CDV NP, a rabbit serum against MV NP [12] that crossreacts with CDV NP and a CDV-infected or normal dog serum. Thereafter, the appropriate second antibodies conjugated with horseradish peroxidase (Organon Technika, Amersham, Buckinghamshire, UK) were applied. Peroxidase activities were detected using the Enhanced Chemiluminescence Western Blotting Detection System (Amersham, Buckinghamshire, UK). The results using the MAb c5 are shown in Fig. 3. When the transferred membranes of transfected or CDV-infected cells were incubated with the two MAb or the antisera against MV NP (data not shown), one major band corresponded to NP was specifically identified at the position of 65 kilodaltons (kd) (Fig. 3, arrowhead). When the membranes were incubated with the serum of a CDV-infected dog, minor bands which did not correspond to the NP were identified only in the cells infected with CDV (data not shown). On the other hand, the same results were obtained in COS cells transfected with pRV-ON (data not shown). However, no virus antigen was detected by IFA or Western blotting in the COS cells.

Fig. 2. Expression of NP protein in Vero cells. Vero cells infected with the Onderstepoort strain of CDV were the positive control and harvested at 11 hr post-infection (B and E). Vero cells were transfected with pRV-ON (C and F), or pRVSV (A and D) then fixed with acetone 48 hr later. NP expression was visualized by indirect immunofluorescence, using anti-CDV NP MAb (f5 for A to C and c5 for D to F), followed by an incubation with anti-mouse IgG conjugated to fluorescein isothiocyanate.
Fig. 3. Western blots of cells expressing NP protein. The lysate of Vero cells infected with the Onderstepoort strain of CDV was the positive control (lane 1). The negative control was the lysate of Vero cells transfected with pRVSV (lane 2). The lysate of Vero cells transfected with pRV-ON is shown in lane 3. The lysates were denatured, resolved by electrophoresis, blotted and sequentially reacted with anti-CDV mouse MAb (c5, ascites, 1:5,000 dilution) which recognizes NP of CDV and incubated with anti-mouse IgG conjugated to horseradish peroxidase. The peroxidase activity was detected using the Enhanced Chemiluminescence Western Blotting Detection System (Amersham, Buckinghamshire, UK). The migration and molecular weight of the markers are indicated on the left. The arrowhead indicates 65 kd NP.

infected with CDV, probably because the virus could not replicate in these cells. Few cell lines are susceptible to CDV infection [6, 10].

In this study, the pRV-ON was demonstrated to be translated and produced intact NP in eucaryotic cells. Localization of the NP in transfected cells is of interest. The NP accumulated in the nuclei of the cells transfected with pRV-ON, whereas it is abundant in the cytoplasm of the cells infected with CDV. This distribution has also been identified in the NP of MV, another morbillivirus and it might be correlated with the consistent nuclear localization of some morbillivirus RNPs as nuclear inclusion bodies [5]. The cellular localization of NP might be due to specific interaction with other viral protein in infected cells, such as phosphoprotein [5]. A further expression study of the NP gene as well as with other viral protein genes of CDV should provide a better understanding of the role of virus protein interaction.

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