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Expression of Sendai Virus Nucleocapsid Protein in a Baculovirus Expression System and Application to Diagnostic Assays for Sendai Virus Infection

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The most common diagnostic technique for the detection of Sendai virus infection in rodents is serological evaluation by enzyme-linked immunosorbent assay (ELISA) with semipurified preparations of whole virions as antigens. This assay often suffers from a lack of specificity. The goal of the present project was to develop more specific antigens for use in diagnostic testing by producing recombinant antigens in insect cells. To identify viral proteins immunoreactive in multiple laboratory rodent species, Western blots (immunoblots) of viral polypeptides were probed with immune sera from mice, rats, and hamsters. The nucleocapsid protein (NP) reacted with immune sera from all species tested. Therefore, the NP gene was selected for cloning and expression in a baculovirus. To construct the recombinant, complementary DNA was synthesized by reverse transcription PCR from Sendai virus RNA with primers from the 5 and 3 termini of the NP-coding region. Amplified DNA was cloned into a baculovirus transfer vector (pBlueBacHis A) and was cotransfected with wild-type baculovirus into insect cells. Baculovirus recombinants containing the NP gene were identified by PCR. Evaluation of the recombinant proteins expressed in insect cells by Western blot analysis revealed specific reactivity with immune sera. In comparison with conventional ELISAs that use whole virions as the antigen, ELISAs that use recombinant NP were more specific.

Sendai virus, a parainfluenza virus of the paramyxovirus family, has a linear single-stranded RNA genome with negative polarity (5) and commonly infects laboratory mice, rats, and hamsters (2, 4). In mice, acute epizootics occur frequently in previously uninfected colonies and are characterized by respiratory distress, neonatal mortality, retarded growth, and prolonged gestation. Susceptible adults exhibit anorexia, hunched posture, and a rough hair coat. Weight loss and dyspnea may also occur (8). In rats and hamsters, Sendai virus infection is usually subclinical, but it may be manifested as ruffled fur, dyspnea, or anorexia (4). Sendai virus infection can induce necrosis of airway epithelium, bronchitis, and in severe cases, bronchopneumonia (1, 4).

Serological methods, such as the enzyme-linked immunosorbent assay (ELISA), are commonly used to determine whether laboratory animals are infected with Sendai virus (4, 7). Sendai virus has been propagated in many cell lines such as LLCMK2 and BHK21, but viral yields in in vitro cell culture systems are poor. Maximum virus production is achieved by infection of embryonated chicken eggs. Unfortunately, this process is labor intensive, and purified virus preparations may remain contaminated with egg proteins, resulting in false-positive reactions when these viral preparations are used as antigens in serological tests (11).

Recently, baculovirus expression systems have been developed. These systems are capable of expressing large amounts of cloned, recombinant proteins in insect cells. In the study described here, cDNA specific for the nucleocapsid protein (NP) of Sendai virus was produced by reverse transcription PCR (RT-PCR) and was cloned into a baculovirus transfer vector. The recombinant transfer vector was cotransfected with wild-type baculovirus, *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV), into insect cells. Recombinant baculoviruses were identified, virus-specific antigen was expressed, and the production of recombinant antigen was optimized. The sensitivities and specificities of ELISAs with recombinant antigen were evaluated and compared with those of conventional ELISAs that use whole virions as the antigen.

MATERIALS AND METHODS

Viruses and cells. Sendai virus (Cantell strain), which was obtained from the American Type Culture Collection, Rockville, Md., was propagated in the allantoic fluid of embryonated chicken eggs and was harvested as described previously (6).

Wild-type AcMNPV, linear AcMNPV, and the baculovirus transfer vector, pBlueBacHis A, were obtained from Invitrogen Corporation (San Diego, Calif.). The virus was propagated in *Spodoptera frugiperda* Sf-9 or High Five insect cell lines (Invitrogen Corp.) at 27°C in insect cell medium (Ex-cell 401; JRH Biosciences, Lenexa, Kan.) with 10% serum supplement (Serum-plus; JRH Biosciences).

Identification of immunodominant Sendai virus antigen. Sendai virus preparations (25 μg) were subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and were electrophoretically transferred to nitrocellulose membranes. Analogous preparations from mock-infected embryonated eggs served as negative controls. Membranes were blocked with 5% nonfat dry milk in phosphate-buffered saline (PBS) and were probed with sera from mice, rats, and hamsters shown to have antibodies to Sendai virus by prior serological testing at the University of Missouri Research Animal Diagnostic and Investigative Laboratory (Columbia, Mo.). Bound antibodies were detected by sequential incubation with biotinylated antispecies secondary antibodies (Vector Laboratories, Burlingame, Calif.), 1% horse serum (Hazelton, Lenexa, Kan.) diluted in PBS, and the Vectastain ABC kit (Peroxidase standard PK-4000; Vector Laboratories). Blots were developed with the TMB membrane peroxidase substrate system (Kirkegaard & Perry Laboratories, Gaithersburg, Md.). Colorimetric reactions were terminated by exhaustive rinsing in deionized water.

Primers. The NP gene, coding for the immunodominant Sendai virus protein, was selected for DNA amplification and subsequent cloning. The primers for the NP gene, SNP1 and SNP2, were designed with *Bam*HI and *Eco*RI sites on the 5′

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TABLE 1. Primer sequences

Primer	Sequence (5′→3′)[a]
Sendai virus	
SNP1	CCG AAT TCG GAT CCC TAG ATT CCT CCT ACC CCA
	*Eco*RI *Bam*HI
SNP2	CCG AAT TCG GAT CCA TGG CCG GGT TGT TGA GCA
	*Eco*RI *Bam*HI
Baculovirus	
Forward primer	TTT ACT GTT TTC GTA ACA GTT TTG
Reverse primer	CAA CAA CGC ACA GAA TCT AG

[a] Underscoring indicates restriction enzyme sites within primers.

termini (Table 1). The foward primer and the reverse primer (Table 1), which flank the polyhedrin gene on pBlueBacHis A and AcMNPV, were used in PCR assays to assess the sizes of the inserts in putative recombinant clones.

RNA extraction and RT-PCR. RNA was isolated from Sendai virus by incubating 0.5 ml of the egg-propagated virus preparation with a final concentration of 2% (wt/vol) SDS and 250 μg of proteinase K per ml for 5 min at 50°C. Viral RNA was purified by phenol-chloroform extraction and ethanol precipitation, and the RNA was suspended in 300 μl of diethyl pyrocarbonate-treated water (3). RT-PCR was performed with the SuperScript preamplification system (GIBCO BRL, Life Technologies, Grand Island, N.Y.) according to the manufacturer's recommendations. For RT, 13 μl of template RNA was annealed with 2 pmol of SNP2 in a total volume of 14 μl at 70°C for 10 min; the mixture was then chilled on ice for 1 min. RT reagents were then added to give a total volume of 20 μl containing synthesis buffer (20 mM Tris-HCl [pH 8.4], 50 mM KCl, 2.5 mM $MgCl_2$, 0.1 μg of bovine serum albumin per μl), 0.5 mM (each) deoxynucleoside triphosphates (dATP, dCTP, dGTP, and dTTP), 10 mM dithiothreitol (DTT), and 200 U of SuperScript II reverse transcriptase. This mixture was incubated at room temperature for 10 min, 42°C for 50 min, and 70°C for 15 min and was then chilled on ice. To remove the RNA templates from the RNA-DNA hybrids, 2 U of *Escherichia coli* RNase H was added to the RT product and the mixture was incubated at 37°C for 20 min. For PCR following RT, a total volume of 100 μl containing synthesis buffer, 0.02 μM (each) primer (SNP1 and SNP2), 21 μl of the reverse-transcribed product, and 5 U of *Taq* polymerase (Boehringer Mannheim, Indianapolis, Ind.) was overlaid with 100 μl of mineral oil. Amplification was performed in a DNA thermal cycler (model 480; Perkin-Elmer, Norwalk, Conn.) as follows. Samples were initially heated at 94°C for 5 min; this was followed by 30 cycles consisting of denaturation (94°C, 1 min), primer annealing (53°C, 1 min), and extension (72°C, 4 min). Electrophoresis of the RT-PCR products was performed through a 3% NuSieve agarose gel (FMC Bioproducts, Rockland, Maine) at 80 V for 2.5 h. The DNA was stained with ethidium bromide and was visualized on a UV transilluminator.

Construction of recombinant virus. Standard DNA manipulation techniques were used to construct recombinant plasmids (9). The amplified PCR product was cleaved with *Bam*HI (Gibco BRL), resolved in a 1% agarose gel, and purified with a Gene-Clean II kit (Bio 101, Inc., La Jolla, Calif.). The baculovirus transfer vector pBlueBacHis A (Invitrogen Corp.) was digested with *Bam*HI and was dephosphorylated with 0.5 U of shrimp alkaline phosphatase (SAP; United States Biochemical, Cleveland, Ohio) at 37°C for 30 min; this was followed by heat inactivation at 65°C for 30 min. The digested cDNA was ligated into the SAP-treated *Bam*HI-digested vector and was used to transform *E. coli*. Ampicillin-resistant colonies were subjected to restriction enzyme analysis with *Bam*HI and *Pst*I to identify clones possessing an insert of the desired size and in the appropriate orientation. Recombinant plasmids containing the desired insert were propagated in *E. coli* and were purified with *Plasmid* SELECT-250 columns (5 Prime → 3 Prime, Inc., Boulder, Colo.) according to the manufacturer's recommendations.

Transfection of recombinant viruses into insect cells was performed with the Transfection Module (Invitrogen Corp.) according to the manufacturer's recommendation, with the exception that Sf-9 cells were used only in the initial transfection; High Five cells were used for plaque purification and production of recombinant protein. Briefly, to obtain recombinant baculovirus that expressed the Sendai NP gene, 2×10^6 Sf-9 cells were transfected with a mixture of 1 μg of linear AcMNPV DNA and 3 μg of purified recombinant pBlueBacHis A by cationic liposome-mediated transfection. Plaques containing putative recombinant viruses were identified by selecting nonoccluded plaques.

PCR analysis of baculovirus recombinants. PCR with primers flanking the polyhedrin gene was performed to confirm that the recombinant clones possessed the NP insert. Briefly, putative recombinant viruses were propagated in High Five cells and viral DNA was isolated from cell-free supernants. An equal volume of 20% polyethylene glycol (PEG 8000) in 1 M NaCl was added to the cell-free supernatant, and the mixture was incubated at room temperature for 30 min. After the mixture was centrifuged at 15,000 × *g* for 10 min at room temperature, the pellet was suspended in 100 μl of sterile water containing 1 mg of proteinase K per ml and the mixture was incubated at 50°C for 1 h. Viral DNA was purified by phenol-chloroform extraction and ethanol precipitation and was resuspended in 10 μl of sterile water. PCR was carried out in a total volume of 100 μl containing 1 μl of viral DNA, 0.5 μg of forward primer, 0.5 μg of reverse primer, 200 μM (each) deoxynucleoside triphosphate (dCTP, dGTP, dATP, and dTTP; Boehringer Mannheim), and PCR buffer (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl [pH 8.3]). The samples were heated to 94°C for 5 min; this was followed by 30 cycles consisting of denaturation (94°C, 1 min), primer annealing (53°C, 1 min), and extension (72°C, 4.5 min). The PCR products were analyzed by agarose gel electrophoresis, stained with ethidium bromide, and visualized under UV light.

Western blot analysis of infected cell polypeptides. To confirm that recombinant clones containing NP gene sequences expressed immunoreactive NP, Western blot (immunoblot) analyses were performed. High Five cells (5×10^5) were infected with NP gene recombinant baculovirus at a multiplicity of infection (MOI) of 10 and were harvested at 3 days postinfection. The cells were rinsed with PBS, suspended in 900 μl of hypobuffer (20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid]–KOH [pH 7.5], 5 mM KCl, 7.5 mM $MgCl_2$, 0.1 mM DTT) with 0.1 mg of protease inhibitor (Pefabloc; Boehringer Mannheim) per ml, incubated on ice for 20 min, and then sonicated on ice for 3 min. The suspension was transferred into a 1.5-ml microcentrifuge tube containing 90 μl of 3 M KCl; the mixture was incubated on ice for 30 min and centrifuged at 17,140 × *g* for 10 min at 4°C. The supernatant was collected and analyzed by Western blot analysis as described above.

Optimization of recombinant protein expression. To determine the optimal conditions for expression of NP by baculovirus-infected cells, High Five cells were infected with NP-recombinant virus at various MOIs and were harvested at various times postinfection. Cell lysates were evaluated by Western blot analyses. Briefly, High Five cells (5×10^5) were seeded into a 12-well tissue culture plate containing 3 ml of Ex-cell 401 with 10% Serum-plus. Three wells each were infected with recombinant virus at an MOI of either 5, 10, or 20, and the contents of one well containing cells with virus at each MOI were harvested at 24, 48, and 72 h postinfection. As controls, one well was infected with wild-type baculovirus at an MOI of 10, and one well was uninfected. Control wells were harvested at 72 h postinfection. The harvested cells were treated with hypobuffer as described above, and 10 μg of extract was subjected to Western blot analysis as described above.

Preparation of recombinant NP for ELISAs. High Five cells infected with recombinant virus at an MOI of 10 were harvested at 72 h postinfection. Cell pellets were resuspended in PBS, frozen and thawed three times, and centrifuged at 17,140 × *g* for 10 min at 4°C. The supernatant was collected and was stored at −70°C.

ELISA. ELISAs with recombinant NP as the antigen (rNP ELISA) and ELISAs with whole Sendai virions harvested from infected embryonated chicken eggs as antigens (SV ELISA) were performed to compare the sensitivity and specificity of each antigen for detection of Sendai virus antibodies. For the rNP ELISA, microtiter plates (Pro-Bind Assay Plate; Falcon, Lincoln Park, N.J.) were incubated at 4°C for 48 h with 200 μl (per well) of recombinant NP or AcMNPV-infected High Five cell extract at a protein concentration of 4 μg/ml (for mouse serum samples) or 2 μg/ml (for rat and hamster serum samples) in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, 3.1 mM NaN_3 [pH 9.6]). For the SV ELISA, microtiter plates were incubated at 4°C for 48 h with 200 μl (per well) of whole Sendai virus or mock-infected egg extract at a protein concentration of 0.5 μg/ml in coating buffer. Prior to use, each well was blocked with 200 μl of 0.5% nonfat dry milk in PBS–Tween 20 (140 mM NaCl, 15 mM NaH_2PO_4, 11 mM Na_2HPO_4, 2.7 mM KCl, 3.1 mM NaN_3, 0.05% Tween 20 [pH 7.4]) at room temperature for 30 min. Serum samples were diluted 1:20 in 0.5% nonfat dry milk in PBS–Tween 20; 200 μl of diluted serum was added in duplicate to wells containing recombinant NP, AcMNPV-infected High Five cell extract, whole virion protein, and mock-infected egg extract; and microtiter plates were incubated at 37°C for 2 h. After the wells were washed three times with plate wash solution (154 mM NaCl, 0.05% Tween 20, 0.0001% thimerosal), 200 μl of alkaline phosphate-conjugated, affinity-purified antispecies secondary antibody diluted in PBS–Tween 20 was added to the wells and the plates were incubated at 37°C for 2 h. After the plates were washed five times with plate wash solution,

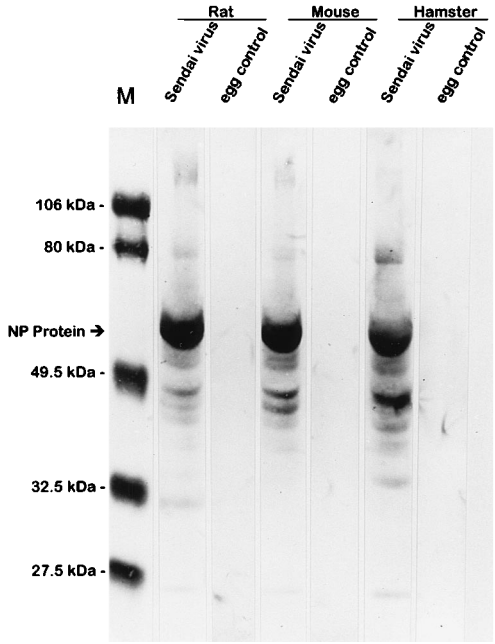

FIG. 1. Identification of immunodominant Sendai virus antigen. Sendai virus proteins were analyzed by SDS-PAGE and Western blot analyses. Protein extracts of mock-infected embryonated eggs served as negative controls. Blots were probed with antisera from mice, rats, and hamsters. Lane M, molecular size markers.

100 μl of *p*-nitrophenyl phosphate (Sigma, St. Louis, Mo.) was added to each well at a concentration of 1 mg/ml in substrate solution (2.1 mM $MgCl_2$, 28 mM Na_2CO_3, 23 mM $NaHCO_3$ [pH 9.8]). After a 1-h incubation at room temperature, the reaction was terminated by the addition of 100 μl of 3 M NaOH to each well, and the absorption was measured at 405 nm. The average of two absorbance values for AcMNPV-coated wells was subtracted from the average of two absorbance values for recombinant NP-coated wells to give an rNP ELISA value that reflected the reactivities of Sendai virus NP-specific antibodies. Similarly, an SV ELISA value was obtained by subtracting the average of two absorbance values for mock-infected egg extract-coated wells from the average of two absorbance values for Sendai virus-coated wells. Cutoff values for the SV ELISA for each species were established previously by evaluating more than 50 negative serum samples from each species by previous SV ELISA. Sera with SV ELISA values greater than the mean plus 2 standard deviations of the absorbance for known negative sera were considered positive.

RESULTS

Identification of immunodominant Sendai virus antigen. To identify the Sendai virus antigen which is immunodominant in all susceptible laboratory rodents, Sendai virus proteins extracted from virions were analyzed by Western blot analyses. Blots were probed with sera from mice, rats, and hamsters that were previously shown to possess antibodies to Sendai virus by SV ELISA. On the basis of the intensity of the staining reactions on Western blot analyses, the 60-kDa NP appeared to be the dominant antigenic Sendai virus protein in the three animal species evaluated (Fig. 1). Therefore, NP was selected for subsequent cloning in a baculovirus expression vector.

Construction of recombinant viruses. RT-PCR was performed with the primers designed to be specific for the 5′ and 3′ termini of the NP gene (10) to synthesize cDNA from NP RNA. Electrophoretic analysis indicated that the RT-PCR product was the expected 1,610 bp in size (data not shown).

To generate NP-recombinant baculoviruses, purified *Bam*HI-digested cDNA was ligated into the *Bam*HI site of the pBlueBacHis A baculovirus transfer vector, which preserved the reading frame of the NP gene product (Fig. 2). The recombinant baculovirus vector possessing the cDNA insert in the appropriate orientation was identified by restriction enzyme analysis and was cotransfected with wild-type AcMNPV into Sf-9 insect cells. Sixteen putative recombinant virus clones were selected, plaque purified, and evaluated by PCR analysis to assess whether the clones possessed the NP insert. Of the 16 clones analyzed, two recombinant clones (clones 1 and 2) were found to contain inserts of the appropriate size (data not shown).

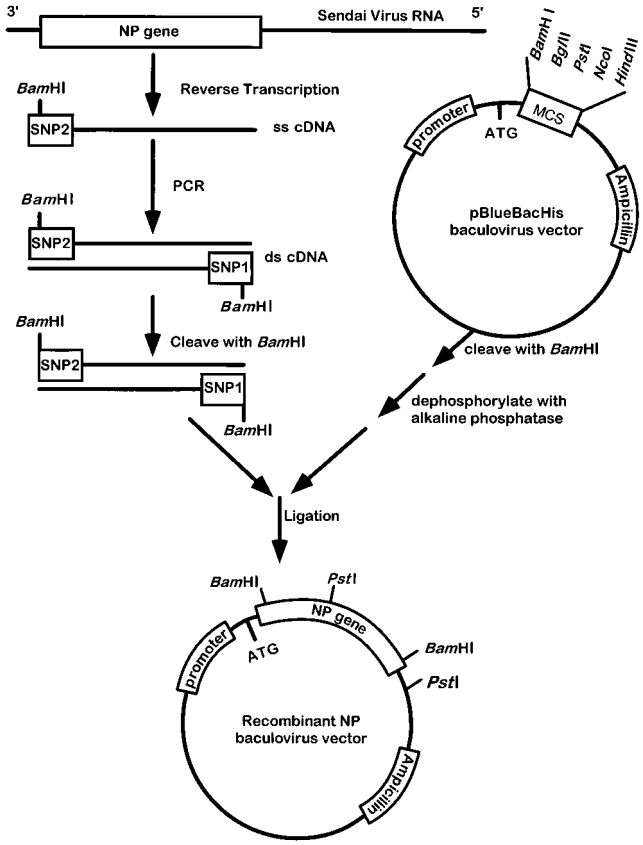

FIG. 2. Construction of recombinant vector. Sendai virus RNA and primers specific for the 5′ and 3′ termini of the NP gene (SNP1 and SNP2) were used in RT-PCR assays to synthesize cDNA corresponding to the Sendai virus NP gene. The product was cleaved with *Bam*HI and was ligated into the dephosphorylated *Bam*HI-digested pBlueBacHis A baculovirus vector to construct a recombinant baculovirus transfer vector. MCS, multiple cloning site; ss cDNA, single-stranded cDNA; ds cDNA, double-stranded cDNA.

Western blot analysis of recombinant NP. SDS-PAGE and Western blot analyses of the polypeptides synthesized by High Five cells infected with clones 1 and 2 were performed to confirm that these recombinant viruses synthesized recombinant NP. The blots were probed with sera from rats previously documented to have antibodies to Sendai virus by SV ELISA. Polypeptides from wild-type baculovirus-infected cells and uninfected cells served as negative controls. The recombinant NPs produced by both clones exhibited a slightly higher molecular weight than that of native NP from Sendai virus (Fig. 3). No immunoreactive peptides were detected in the protein extracts of wild-type baculovirus-infected cells or uninfected cells. NP-recombinant virus clone 1 was selected for subsequent studies.

Optimization of recombinant NP expression. To optimize the production of recombinant NP by infected insect cells,

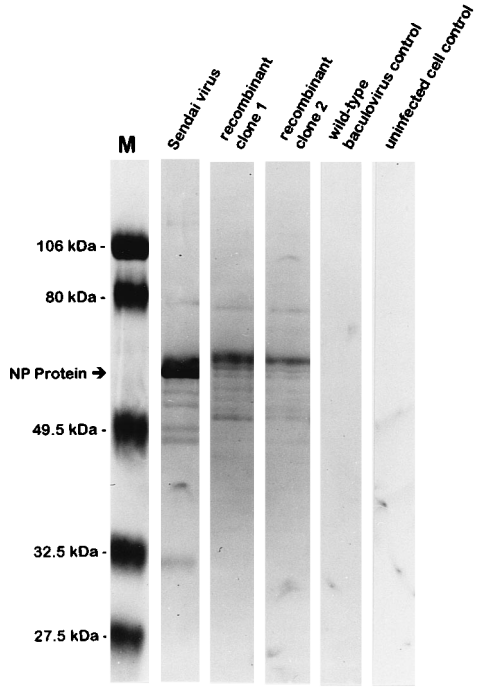

FIG. 3. Western blot analysis of recombinant NP. High Five cells were infected with clones which were positive for NP sequences by PCR and were harvested at 3 days postinfection. Western blots were probed with sera from rats with Sendai virus antibodies. Protein extracts from wild-type baculovirus-infected cells and uninfected cells served as negative controls. Lane M, molecular size marker.

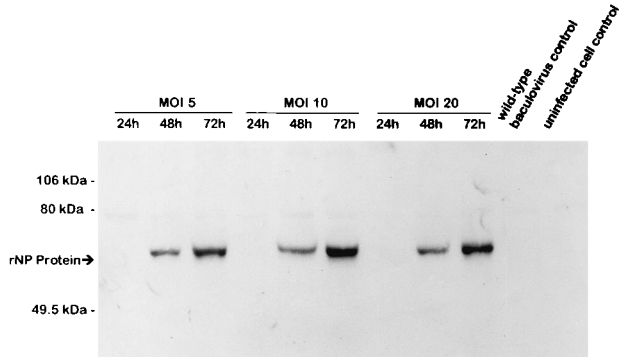

FIG. 4. Optimization of recombinant NP expression. High Five cells were infected with NP-recombinant virus at various infection doses (MOIs, 5, 10, and 20) and various incubation periods of infection (24, 48, and 72 h postinfection). Protein extracts were analyzed by Western blot analyses. Western blots were probed with sera from rats with Sendai virus antibodies. Protein extracts from wild-type baculovirus-infected cells and uninfected cells served as negative controls.

various infection doses (MOIs, 5, 10, and 20) and various incubation periods of infection (24, 48, and 72 h postinfection) were evaluated by Western blot analysis for recombinant NP synthesis in High Five cells infected with NP-recombinant virus (Fig. 4). Uninfected cells and cells infected with wild-type virus (MOI, 10) for 72 h served as negative controls. No recombinant NP was detected at 24 h postinfection at any of the three infection doses. At 48 h postinfection, recombinant protein was detected at all three MOIs; the amount of recombinant protein produced appeared to be similar among cells receiving the various infection doses. Maximum production of recombinant NP was detected at 72 h postinfection. On the basis of the visual intensity of the immunoreactive band, it appeared that a slightly larger amount of recombinant protein was produced at an MOI of 10 compared with the amount produced at an MOI of 5 or 20. No immunoreactive proteins were detected in the protein extracts of wild-type baculovirus-infected cells or uninfected cells.

ELISA. ELISAs were performed to evaluate the antigenicity of recombinant NP produced by the baculovirus expression system. The sensitivities and specificities of the ELISAs with the recombinant NP antigen were found to be optimal when the microtiter plates were coated with 4 μg of antigen per ml for mouse serum samples and 2 μg of antigen per ml for rat and hamster serum samples (data not shown). Cutoff values for the ELISA with recombinant antigens were established for each animal species (mice, rats, and hamsters) by evaluating serum samples from 55 mice, 95 rats, and 29 hamsters which were previously shown to be negative for Sendai virus antibodies by SV ELISA. Sera with mean absorbance values greater than the mean plus 2 standard deviations were considered positive. To evaluate the sensitivity and specificity of the test, an additional 120 mouse, 340 rat, and 40 hamster serum samples were examined in the rNP ELISA and the SV ELISA, and the results obtained from those two ELISAs were compared with each other (Table 2). When discordant results were obtained between rNP ELISAs and SV ELISAs, Western blot analyses with whole Sendai virus proteins or a mock-infected egg preparation as antigens were performed on the sera with discordant ELISA results to clarify the immune status of the samples. Serum samples were considered to be positive for Sendai virus antibodies if blots revealed banding patterns that were consistent with the known sizes of Sendai virus proteins. The sensitivities and specificities of the rNP ELISAs and SV

TABLE 2. Comparison of rNP ELISA with SV ELISA[a]

Immune status[b]	No. of serum samples											
	Mouse serum				Rat serum				Hamster serum			
	rNP ELISA		SV ELISA		rNP ELISA		SV ELISA		rNP ELISA		SV ELISA	
	+	−	+	−	+	−	+	−	+	−	+	−
+	54	2	55	1	210	17	227	0	11	0	11	0
−	2	62	5	59	4	109	15	98	0	29	0	29

[a] For mouse serum the sensitivities of the rNP ELISA and the SV ELISA were 96.4 and 98.2%, respectively; the specificities were 96.9 and 92.2%, respectively. For rat serum the sensitivities of the rNP ELISA and the SV ELISA were 92.5 and 100%, respectively; the specificities were 96.5 and 86.7%, respectively. For hamster serum the sensitivities and specificities of both ELISAs were 100%.

[b] Immune status was confirmed by Western blot analysis of serum samples.

ELISAs were calculated for each species. The results revealed that the rNP ELISA was nearly as sensitive as and was more specific than the conventional SV ELISA for mouse and rat serum samples and that the rNP ELISA was as sensitive and specific as the SV ELISA for hamster serum samples.

DISCUSSION

ELISAs are commonly used to determine whether laboratory animals are infected with Sendai virus. Because of poor virus yields in cell lines, maximum virus production requires infection of embryonated chicken eggs. Propagation of Sendai virus in eggs is very time consuming and labor-intensive, since embryonated eggs must be incubated for 11 days prior to inoculation, the inoculated eggs must be incubated for 2 days postinfection, and then the allantoic fluid must be harvested from individual eggs. Another disadvantage of egg-derived antigens is that purified virus preparations may remain contaminated with egg proteins, resulting in false-positive serological results. The recombinant NP synthesized in the baculovirus expression system was tested as an approach to improving the specificities of ELISAs for the detection of antibodies to Sendai virus and eliminating the tedious procedures associated with the propagation of the virus in embryonated eggs. Once the NP-recombinant baculovirus has been constructed, production of viral antigen with the baculovirus expression system is much easier and less time consuming than that with egg-derived preparations, since large numbers of insect cells can readily be inoculated and harvested within 3 days.

The recombinant NP produced by the baculovirus expression system appeared to be very similar antigenically to the native NP of Sendai virus. By Western blot analysis, the recombinant NP exhibited a slightly higher molecular size (63.8 kDa) than that of the native protein (60 kDa). Recognizing that the recombinant protein was expressed as a fusion protein that included 36 amino acids coded by the 5′ portion of the polyhedrin gene, the increased molecular size of the recombinant product was consistent with the expected size of the fusion protein.

The rNP ELISA required a higher antigen concentration in the ELISAs of mouse serum samples (4 μg/ml) than in those of rat and hamster serum samples (2 μg/ml). Although the reason for this difference is unclear, it is possible that antibodies from different species recognize different epitopes on the NP of Sendai virus and that fewer epitopes were recognized by mouse antibodies. Alternatively, more epitopes recognized by mouse antibodies may have been destroyed during protein preparation.

Some discordant results between the rNP ELISA and the SV ELISA were obtained during evaluation of the antigenicity of the recombinant NP produced by the baculovirus expression system. Western blot analyses were performed to clarify the immune status of these serum samples. Sera were used to probe lanes containing Sendai virus proteins and control lanes containing antigen preparations from uninfected eggs. In the majority of cases, sera that yielded positive results in the SV ELISA and negative results in the rNP ELISA exhibited very weak reactivities to Sendai virus proteins. This suggested that the rNP ELISA was not as sensitive as the SV ELISA. This may be caused by the lack of other antigenic proteins of Sendai virus and the loss of some epitopes of recombinant NP during protein preparation. In other cases, sera that yielded positive results on the SV ELISA and negative results on the rNP ELISA exhibited reactivity to egg proteins but not to Sendai virus proteins. On the basis of these results, we concluded that the rNP ELISA can avoid the false-positive results caused by antibody reactive to egg protein contamination.

In summary, the results of the present study demonstrated that the recombinant Sendai virus NP can be expressed in a baculovirus expression system in insect cells and that the recombinant NP closely resembles the native protein in size and antigenicity. The ELISA developed with the recombinant NP was nearly as sensitive as and was more specific than the conventional Sendai virus ELISA, which uses whole virions as the antigen, for detecting antibodies in mouse and rat sera. For hamster serum samples, the rNP ELISA was as sensitive and as specific as the conventional SV ELISA. On the basis of these results, the baculovirus expression system is a good method for producing Sendai virus NP, which retains its antigenicity and which can be applied as an antigen in ELISAs to detect anti-Sendai virus antibodies in mice, rats, and hamsters.

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