Rescue of Newcastle Disease Virus from Cloned cDNA: Evidence that Cleavability of the Fusion Protein Is a Major Determinant for Virulence

BEN P. H. PEETERS,* OLAV S. DE LEEUW, GUUS KOCH, AND ARNO L. J. GIELKENS
Department of Avian Virology, Institute for Animal Science and Health, 8200 AB Lelystad, The Netherlands

Received 18 November 1998/Accepted 24 February 1999

A full-length cDNA clone of Newcastle disease virus (NDV) vaccine strain LaSota was assembled from subgenomic overlapping cDNA fragments and cloned in a transcription plasmid between the T7 RNA polymerase promoter and the autocatalytic hepatitis delta virus ribozyme. Transfection of this plasmid into cells that were infected with a recombinant fowlpoxvirus that expressed T7 RNA polymerase, resulted in the synthesis of antigenomic NDV RNA. This RNA was replicated and transcribed by the viral NP, P, and L proteins, which were expressed from cotransfected plasmids. After inoculation of the transfection supernatant into embryonated specific-pathogen-free eggs, infectious virus derived from the cloned cDNA was recovered. By introducing three nucleotide changes in the cDNA, we generated a genetically tagged derivative of the LaSota strain in which the amino acid sequence of the protease cleavage site (GGGQGR) of the fusion protein F0 was changed to the consensus cleavage site of virulent NDV strains (GRRQRR) of F. Pathogenicity tests in day-old chickens showed that the strain derived from the unmodified cDNA was completely nonvirulent (intracerebral pathogenicity index [ICPI] = 0.00). However, the strain derived from the cDNA in which the protease cleavage site was modified showed a dramatic increase in virulence (ICPI = 1.28 out of a possible maximum of 2.0). Pulse-chase labeling of cells infected with the different strains followed by radioimmunoprecipitation of the F protein showed that the efficiency of cleavage of the F0 protein was greatly enhanced by the amino acid replacements. These results demonstrate that genetically modified NDV can be recovered from cloned cDNA and confirm the supposition that cleavage of the F0 protein is a key determinant in virulence of NDV.

Newcastle disease is a serious avian disease with worldwide distribution that can cause severe economic losses in the poultry industry (2). The causative agent of the disease, Newcastle disease virus (NDV) or avian paramyxovirus type 1, is a member of the Paramyxoviridae and has been assigned to the genus *Rubulavirus* in the subfamily Paramyxovirinae (31). However, recently we presented evidence suggesting that NDV is not a member of the genus *Rubulavirus* but should be assigned to a new genus within the subfamily *Paramyxovirinae* (11). Similar to other *Paramyxoviridae*, NDV contains a nonsegmented single-stranded RNA genome of negative polarity (24). The RNA is 15,186 nucleotides (nt) in size (11, 23, 38) and contains six genes which encode the nucleocapsid protein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase (HN), and large polymerase protein (L) (30). In addition to these gene products, additional proteins (designated V and W protein) may be produced by an RNA-editing event that occurs during transcription of the P gene (48). NDV lacks the gene encoding the small hydrophobic protein that is occasionally present in members of the genus *Rubulavirus* (24).

NDV strains can be classified as highly virulent (velogenic), intermediate (mesogenic), or nonvirulent (lentogenic) on the basis of their pathogenicity for chickens (5). The molecular basis for pathogenicity of NDV is mainly determined by the amino acid sequence of the protease cleavage site of the F protein and by the ability of cellular proteases to cleave the F protein of different pathotypes (34, 35). Cleavage of the pre-

* Corresponding author. Mailing address: Institute for Animal Science and Health (ID-DLO), Department of Avian Virology, P.O. Box 65, 8200 AB Lelystad, The Netherlands. Phone: 31 320-238238. Fax: 31-320-238668. E-mail: b.p.h.peeters@id.dlo.nl.
acid sequence of the protease cleavage site of the F0 protein, we proved that cleavability of the F0 protein is a key determinant for virulence of NDV.

MATERIALS AND METHODS

Cells and viruses. Primary chicken embryo fibroblasts (CEF) cells and CER cells (47) were grown in Glasgow modification of Eagle’s medium (GMEM)-Eagle’s minimum essential medium (1:1; both from ICN) containing 5% fetal calf serum. QM5 cells (3) were grown in medium 199 supplemented with 10% tryptose phosphate broth and 10% fetal calf serum. BHK-21 cells (ATCC CCL-10) were grown in GMEM containing 5% fetal calf serum. NDV strain LaSota (ATCC VR-699) was plaque purified by three rounds of plaque purification on CEF cells. Virus from the third round of plaque purification (designated clone E13-1) was grown in 9- to 11-day-old embryonated specific-pathogen-free (SPF) eggs. The fowlpox recombinant virus FPV-T7, designated pOLTV5, was constructed from plasmid pOK12 (49) and transcribed in which the T7 DNA-dependent RNA polymerase promoter was deleted to eliminate as much unique restriction sites as possible. Transcription was grown on primary chicken embryo liver cells.

Construction of transcription vector. A low-copy-number transcription vector, designated pOLTV5, was constructed from plasmid pOK12 (49) and transcription vector 2.0 (a generous gift of Andrew Ball) (37) by using standard molecular biological techniques (43). Plasmid pOK12 was digested with PvuI, and the DNA fragment containing the replication origin and the kanamycin resistance gene was isolated. This DNA fragment was ligated to an Eco47III-AflIII fragment (the AflIII site was made blunt by using Klenow DNA polymerase I) from transcription vector 2.0. From the resulting plasmid, an XbaI-Nhel fragment was deleted to eliminate as much unique restriction sites as possible. Transcription vector pOLTV5 contains the T7 DNA-dependent RNA polymerase promoter followed by unique StuI and SmaI restriction sites, the autocalic activity of the hepatitis delta virus, and the transcription termination signal from bacteriophage T7. DNA fragments cloned between the StuI and SmaI restriction sites can be transcribed either in vitro or in vivo by using T7 RNA polymerase. After transcription, the 5' end of the resulting transcripts contains two G residues encoded by the plasmid. Due to the autocatalytic activity of the hepatitis delta virus ribozyme, the 3' end of the transcripts corresponds to the exact terminal nucleotide of the cloned DNA fragment (37).

Construction and cloning of full-length NDV cDNA. Large (4- to 7-kb) subgenomic cDNA fragments spanning the entire NDV genome were generated by high-fidelity RT-PCR as described by de Lecouw and Peeters (11). The cDNA fragments were joined at shared restriction sites and assembled in transcription plasmid pOLTV5 in which the 3'- and 5'-terminal NDV sequences were previously cloned between the StuI and SmaI sites (see text for details). The genetic map of NDV is shown at the top, and the horizontal lines below the genetic map indicate positions of the individual cDNAs. The bottom line shows the final composition of the full-length cDNA.

FIG. 1. Full-length NDV cDNA was assembled from subgenomic overlapping cDNA fragments that were generated by high-fidelity RT-PCR as described by de Lecouw and Peeters (11). The cDNA fragments were joined at shared restriction sites and assembled in transcription plasmid pOLTV5 in which the 3'- and 5'-terminal NDV sequences were previously cloned between the StuI and SmaI sites (see text for details). The genetic map of NDV is shown at the top, and the horizontal lines below the genetic map indicate positions of the individual cDNAs. The bottom line shows the final composition of the full-length cDNA.
(GGRQGR L) to that of the consensus cleavage site for virulent NDV strains (GRRRQR L F). The second PCR fragment was generated by using primers F3F (5'-GGAGACAGTGCAGTTATCAGCGCAATTTG-3') and IV09 (5'-CTCTGTCGACACAGACTACCAGAACTTTCAC-3'). The two overlapping PCR fragments (the overlap is underlined in the primer sequences) were joined in a second PCR by using primers NDV5F and IV09. The resulting fragment, which contains the entire open reading frame of the F gene and encodes a virulent protease cleavage site, was cloned in pCIneo, yielding pCIneoFwt. The StuI-NotI fragment (nt 4646 to 4952) from pCIneoFwT was used to replace the corresponding fragment in subgenomic cDNA clone L21a (Fig. 1), which was subsequently used to regenerate full-length cDNA. The resulting plasmid was designated pNDFLtag (Fig. 2).

**Introduction of a unique HpaI restriction site in full-length NDV cDNA.** Plasmid pNDFLtag (Fig. 2) was digested with AarII (Fig. 2A), and the smaller of the two fragments, which contained almost the entire pOLT5 vector and a small part (226 nt) of the 3' end of NDV, was circularized by using T4 DNA ligase. The resulting plasmid (designated pAAT2) was digested with XmnI, ligated to a nonphosphorylated synthetic 12-mer linker (CCTGTTAACAGG), and recircularized by using T4 DNA ligase. The resulting plasmid (pAAT2HPA), which contained a unique HpaI site (underlined), was digested with AarII and ligated with the larger AarII fragment from pNDFLtag to regenerate full-length NDV cDNA. The resulting plasmid was designated pNDFLtagHPA (Fig. 2A and D).

**RT-PCR and sequence analysis.** The nucleotide sequence of the region of the F gene that encodes the protease cleavage site was analyzed by sequencing an RT-PCR fragment obtained by using primer 3UIT (5'-ACCAAACAGAGAATCCGTGAGTTA-3') for reverse transcription and primers P4731 (5'-AAGCTCCTCCCGAATCTGCC-3') and P5020 (5'-GCGGCAATGCTCTCTTTAA-3') for forward transcription. The positions of the transcription start of T7 RNA polymerase and the cleavage site of the ribozyme are indicated by arrows. The sequence of NDV is boxed. The resulting amino acid changes are underlined and shown in bold. The arrows indicate the position of proteolytic cleavage. (D) Nucleotide sequence in pNDFLtagHPA of the synthetic 12-mer linker (boxed) which was used to insert a unique HpaI restriction site (bold) between nt 109 and 110 (XmnI sites; GAANN NNTTIC) of NDV.

**FIG. 2.** (A) Circular map of the pNDFL plasmids, which consist of full-length NDV cDNA cloned between the StuI and SmaI restriction sites of transcription plasmid pOLT5 (see text for details). Relevant genetic elements and restriction sites are shown. T7, RNA polymerase promoter; rbz, hepatitis delta virus ribozyme; 3’, 3’-terminal leader sequence of NDV; 5’, 5’-terminal trailer sequence of NDV. (B) Nucleotide sequence in plasmid pNDFL of the T7 RNA polymerase promoter, the 3’- and 5’-terminal ends of NDV strain LaSota, and part of the flanking hepatitis delta virus ribozyme. The positions of the transcription start of T7 RNA polymerase and the cleavage site of the ribozyme are indicated by arrows. The sequence of NDV is boxed. (C) Nucleotide and deduced amino acid sequences of the region of the F gene that specifies the protease cleavage site of the F0 proteins in plasmids pNDFL and pNDFLtag. The nucleotide changes introduced to modify the amino acid sequence of the cleavage site are boxed (see also Fig. 3). The resulting amino acid changes are underlined and shown in bold. The arrows indicate the position of proteolytic cleavage. (D) Nucleotide sequence in pNDFLtagHPA of the synthetic 12-mer linker (boxed) which was used to insert a unique HpaI restriction site (bold) between nt 109 and 110 (XmnI sites; GAANN NNTTIC) of NDV.
TABLE 1. HI titers of antisera and MAbs

<table>
<thead>
<tr>
<th>Strain</th>
<th>NDV serum</th>
<th>MAb 7B7</th>
<th>MAb 7D4</th>
<th>MAb 5A1</th>
<th>MAb 4D6</th>
<th>ICPI†</th>
<th>Pathotype‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>LaSota E13-1</td>
<td>256</td>
<td>320</td>
<td>5,120</td>
<td>40</td>
<td>10,240</td>
<td>0.31</td>
<td>Lentogenic</td>
</tr>
<tr>
<td>NDFL</td>
<td>128</td>
<td>2,560</td>
<td>10,240</td>
<td>1,280</td>
<td>10,240</td>
<td>0.00</td>
<td>Lentogenic</td>
</tr>
<tr>
<td>NDFLtag</td>
<td>256</td>
<td>640</td>
<td>10,240</td>
<td>20</td>
<td>10,240</td>
<td>1.28</td>
<td>Mesogenic</td>
</tr>
<tr>
<td>NDFLtagHPA</td>
<td>128</td>
<td>2,560</td>
<td>2,560</td>
<td>640</td>
<td>10,240</td>
<td>1.18</td>
<td>Mesogenic</td>
</tr>
<tr>
<td>Hitchner B1</td>
<td>256</td>
<td>80</td>
<td>10,240</td>
<td>320</td>
<td>10,240</td>
<td>0.11</td>
<td>Lentogenic</td>
</tr>
<tr>
<td>Herts</td>
<td>128</td>
<td>2,560</td>
<td>10,240</td>
<td>1,280</td>
<td>10,240</td>
<td>1.86</td>
<td>Veloegenic</td>
</tr>
<tr>
<td>Texas GB</td>
<td>128</td>
<td>10,240</td>
<td>10,240</td>
<td>1,280</td>
<td>10,240</td>
<td>1.64</td>
<td>Veloegenic</td>
</tr>
</tbody>
</table>

* Expressed as the reciprocal of the highest serum dilution that caused inhibition of hemagglutination. Results for control serum and APMV3 serum were in all cases negative.
† Determined as described by Alexander (1).
‡ Lentogenic strains, ICPI < 0.7; mesogenic strains, ICPI 0.7 to 1.4; velogenic strains, ICPI > 1.4.
strain (Table 1). The virus that was recovered from the inoculated eggs was designated NDFL to distinguish it from the original LaSota strain.

**Generation of genetically modified NDV from full-length cDNA.** To show unambiguously that the cotransfection system could be used to recover infectious virus from cloned full-length NDV cDNA, genetic tags were introduced in plasmid pNDFL. First, the amino acid sequence of the protease cleavage site in the F0 protein was changed from that of the LaSota strain (GGRQGR\(2\)L) to the consensus sequence of virulent NDV strains (GRRQRR\(2\)F) (41) by means of PCR mutagenesis (for details, see Materials and Methods). The resulting plasmid was designated pNDFLtag (Fig. 2A and C). By changing the amino acid sequence of the proteolytic cleavage site of the F gene, which is an important determinant for the virulence of different NDV isolates (14, 34, 41), we aimed at introducing both a genetic tag as well as a phenotypic tag. We made another derivative of pNDFL which contained, in addition to the modified F gene, a unique restriction site as the result of the introduction of a small oligonucleotide into the NDV genome. It has been shown that efficient replication of several paramyxoviruses is dependent on the genome length being a multiple of six nucleotides (so-called rule of six) (8, 22). The genome length of NDV LaSota (15,186 nt) is a multiple of six, which suggests that also replication of NDV is dependent on the rule of six. Therefore, a synthetic 12-mer oligonucleotide linker that contained an HpaI restriction site was introduced in the XmnI site between nt 109 and 110 of NDV in plasmid pNDFLtag. The resulting plasmid was designated pNDFLtagHPA (Fig. 2A and D).

Plasmids pNDFLtag and pNDFLtagHPA were used to generate virus by using the cotransfection system described above. Infectious viruses, designated NDFLtag and NDFLtagHPA, respectively, were recovered from the allantoic fluid of embryonated eggs. In an HI test, all MAbs including 7D4, which is specific for the LaSota strain (26, 29), showed the same reaction pattern with the newly generated viruses as with the original LaSota strain (Table 1). The nucleotide sequence of the region encoding the protease cleavage site of the F protein of NDFLtag was determined by sequencing an RT-PCR fragment that covered the corresponding genome sequence. The results showed that the expected nucleotide sequence was present in the F gene of NDFLtag (Fig. 3). The presence of the unique HpaI restriction site in NDFLtagHPA was verified by means of RT-PCR followed by HpaI digestion. As shown in Fig. 4, the PCR fragment from strain NDFLtagHPA was cleaved by HpaI and generated two fragments of the expected sizes. These results unambiguously show that NDFLtag and NDFLtagHPA were derived from plasmids pNDFLtag and pNDFLtagHPA,
respectively, and demonstrate that genetically modified NDV can be generated from cloned full-length NDV cDNA.

The protease cleavage site of the F protein of NDV is a key determinant for virulence. It is generally assumed that the amino acid sequence of the protease cleavage site of the F0 protein is an important determinant for virulence of NDV strains (14, 34, 41). However, since other differences in the genomic sequence of different NDV strains may contribute to virulence (27, 28), definite proof is still lacking. The availability of strains NDFL and NDFLtag, which differ only in the amino acid sequence of the protease cleavage site of the F0 protein, offered the unique opportunity to test this supposition. The virulence of the original LaSota strain (clone E13-1) and of the newly generated strains NDFL, NDFLtag, and NDFLtagHPA was examined by determining the ICPI in day-old chickens. The results showed that the ICPI of strains NDFLtag and NDFLtagHPA were 1.28 and 1.18, respectively, and demonstrate that genetically modified NDV can be generated from cloned full-length NDV cDNA.

In this report we describe the generation of infectious NDV entirely from cloned full-length cDNA. This was accomplished by using an approach in which cDNA-encoded antigenomic RNA is synthesized by means of T7 RNA polymerase in cells that simultaneously express the viral replication proteins NP, P, and L from cotransfected plasmids. A similar approach has been used to generate infectious virus from cloned full-length cDNA of a number of other nonsegmented negative-strand RNA viruses (4, 7, 9, 12, 13, 17, 19, 20, 25, 40, 45, 50). In accordance with the results of others (13, 17, 19, 25, 45), we observed that the addition of two extra G residues at the 5′ end of the antigenomic RNA by T7 RNA polymerase did not prohibit the formation of infectious virus. Analysis by means of rapid amplification of the 5′ end of viral RNA of the cDNA-derived strains showed that the two G residues were lost during replication (data not shown).

The cotransfection system that we developed for NDV seems to be very efficient. By using approximately 1 μg of total DNA (0.25 μg of pNDFL, 0.4 μg of pClneoNP, 0.2 μg of pClneoP, and 0.2 μg of pClneoL) per 3.5-cm-diameter culture dish, we can generate tens of infective centers in transfected monolayers (data not shown). Inoculation of a fraction (5 to 10%) of the transfection supernatant into embryonated eggs results in virus multiplication that can be easily quantitated by means of a hemagglutination assay. The addition of allantoic fluid to the culture medium of transfected cells was required for the successful recovery of infectious NDV. Allantoic fluid of embryonated eggs contains specific proteases that are absent from cultured cells and which are needed to cleave the F0 protein of lentogenic NDV strains (34, 35). Cleavage of the F0 protein into the F1 and F2 subunits is required to liberate a highly hydrophobic fusion domain at the N terminus of F1 that is required for the membrane fusion activity of the F protein (41). We expected that the addition of allantoic fluid to the transfection supernatant would not be necessary since the virus would be activated after inoculation of the transfection supernatant into the allantoic cavity of embryonated eggs. However, attempts to rescue infectious virus from transfection supernatants that lacked allantoic fluid were unsuccessful. This observation suggests that cleavage of the F protein is required for virus release. However, since the amount of virus that is released from the transfection monolayer will be much larger in the presence of allantoic fluid, this observation may merely indicate that successful recovery of virus from inoculated eggs is dependent on the amount of infectious virus in the inoculum.

The viral RNA that was used to synthesize cDNA was derived from plaque isolate E13-1 of NDV vaccine strain LaSota. Clone E13-1 was obtained after three rounds of plaque purification and was passaged in embryonated eggs before viral RNA was isolated. Nevertheless, the nucleotide sequence of the NDV cDNA in plasmid pNDFL differed at nine positions from the sequence that was determined for clone E13-1 (11). These differences are probably the result of misincorporation during reverse transcription and/or PCR amplification, not of strain variations. Despite these differences, the cDNA could be used to generate infectious virus (designated strain NDFL). When we tested the virulence of NDV by intracerebral inoculation of day-old chickens, it proved to be virtually nonvirulent (ICPI = 0.00 [Table 1]). Since virulence was lower than that of the parent virus clone E13-1 (ICPI = 0.31), it seems...
that one or several of the nucleotides that differ between NDFL and clone E13-1 are responsible for the difference in virulence. In this respect, it is worthwhile mentioning that plaques produced on CEF cells by strain NDFL were somewhat smaller in size than plaques produced by strain E13-1 (Fig. 6). This difference in plaque size was more pronounced when the cells were grown under an agarose overlay than when they were grown under a liquid overlay. Furthermore, NDFL and E13-1 reached similar titers when they were grown in embryonated eggs ($1.4 \times 10^9$/ml for E13-1 and $1.2 \times 10^9$/ml for NDFL, respectively), indicating that replication of strain NDFL is not impaired in embryonated eggs. Since NDFL is nonvirulent, it will be interesting to test its capacity as a live vaccine against Newcastle disease.

That genetic modification is a powerful tool to study the biological functions of viral gene products is demonstrated by our observation that modifying the protease cleavage site of the F0 protein can dramatically change the virulence of NDV. Amino acid sequencing of the F0 precursor proteins of many NDV strains has shown that lentogenic viruses have a single arginine (R) that links the F2 and F1 chains, whereas mesogenic or velogenic strains possess additional basic amino acids forming two pairs at the site of cleavage. Furthermore, the F2 chain of virulent strains generally starts with a phenylalanine (F) residue whereas that of nonvirulent strains generally starts with a leucine (L) (41). When the protease cleavage site of the F0 protein of strain NDFL (GGRQGR↓L) was converted to the consensus protease cleavage site of virulent NDV strains...

FIG. 6. Plaques produced by NDV strains E13-1 (A) and NDFL (B) on CEF cells. CEF cells were infected with virus and incubated for 5 days under a 1% agarose overlay containing 5% allantoic fluid. Plaques were visualized by immunological staining by using a MAb against the NDV F protein.
This modification resulted in a dramatic increase in virulence from ICPI = 0.00 for NDFL to ICPI = 1.28 (out of a possible maximum of 2.0) for NDFLtag (Table 1). Thus, the virulence of NDFLtag is similar to that of a mesogenic strain whereas the antigenic profile is similar to that of the lentogenic parent strain LaSota (Table 1). That the genetic modification indeed affected cleavage of the F0 protein was verified by performing pulse-chase experiments followed by radioimmunoprecipitations, which showed that the efficiency of cleavage of the F0 protein was greatly enhanced by the amino acid replacements (Fig. 5). These results provide the definite proof that the efficiency of cleavage of the F0 protein is the key determinant for virulence of NDV. By using the same approach, the cleavage site of the F0 protein may be modified, at will, to any other amino acid sequence. This may lead to the generation of a series of NDV strains that display a spectrum of virulence levels in vivo.

Our results indicate that the efficiency of cleavage of the F0 protein is not the only determinant that is responsible for virulence of NDV. Velogenic NDV strains may exhibit an ICPI as high as the maximum possible value of 2.0 (2). This indicates that, apart from the cleavage site of the F0 protein, additional nucleotide sequences in the genomic RNA of NDV contribute to virulence. For instance, differences in transcription and translation may modulate growth and cell-to-cell spread of the virus and/or cytopathogenicity (27, 28). The availability of an infectious cDNA of NDV allows for the systematic analysis of sequences that are involved in transcription and replication. This may lead to the design of new NDV vaccines that combine optimal immunogenicity with complete safety.

Apart from the modification of the cleavage site of the F0 protein, a genetic tag consisting of an HpaI restriction site was introduced in the NDV cDNA in order to facilitate the identification of infectious virus that is generated from cloned cDNA. Since replication of several paramyxoviruses (probably including NDV) is dependent on the genome length being a multiple of 6 nt (8, 22), we inserted 12 nt in the NDV genome between nt 109 and 110 (Fig. 2D). This particular position was chosen because it had been shown for Sendai virus that an insertion of a short oligonucleotide linker was tolerated at an analogous position close to the start of the NP gene but not at a position more upstream (16). Furthermore, it has been shown that the genomic and antigenic promoters of the paramyxovirus simian virus 5 consist of two discontinuous elements, designated conserved region I, comprising the terminal 19 nucleotides, and conserved region II, comprising nt 73 to 90 from the terminal end (32, 33). The successful generation of recombinant virus NDFLtagHPA indicates that the insertion of 12 nt at a position close to but beyond the putative genomic promoter is well tolerated and apparently does not interfere with replication. The virulence of strain NDFLtagHPA (ICPI = 1.18) was close to that of its parent strain NDFLtag (ICPI = 1.28), indicating that the insertion did not greatly influence the virulence of the recombinant virus. Furthermore, plaques produced by NDFLtagHPA and NDFLtag on CEF were similar in size, and the two strains reached similar virus titers after growth in embryonated eggs.

Safety is one of the most important properties of live vaccines. However, for many live vaccines, including NDV, immunogenicity is often inversely related to virulence. Therefore, further attenuation of live vaccines without losing immunogenicity is one of the most desired alterations for which genetic modification could be used. In this respect, it has been shown that elimination of expression of the V protein of Sendai virus resulted in a markedly reduced in vivo pathogenicity for mice (21). Similar to Sendai virus, NDV generates a V protein from the P gene by a mechanism known as RNA editing (48). It is conceivable that elimination of expression of the V protein of NDV may also result in an attenuated phenotype in vivo.

Several properties make NDV an attractive vaccine vector for vaccination of poultry against respiratory or intestinal diseases. (i) NDV can be easily cultured to very high titers in embryonated eggs. (ii) Mass culture of NDV in embryonated eggs is relatively cheap. (iii) NDV vaccines are relatively stable and can be simply administered by mass application methods such as addition to drinking water or by spraying or aerosol formation. (iv) The natural route of infection of NDV is by the respiratory and/or intestinal tract, which are also the major natural routes of infection of many other poultry pathogens. (v) NDV can induce local immunity despite the presence of circulating maternal antibody. Since other paramyxoviruses have successfully been used for the incorporation and expression of foreign genes (16, 17, 46), we expect that NDV can also be used as a vaccine vector for the delivery of foreign antigens to the immune system.

ACKNOWLEDGMENTS

We thank Francis Balk for the inoculation of eggs and for performing hemagglutination and HI tests. We thank Michael Skinner for providing the fowlpox-T7 recombinant virus and Andrew Ball for providing transcription plasmid 2.0.

Part of this research was sponsored by Lohmann Animal Health GmbH & Co. KG, Cuxhaven, Germany.

REFERENCES


