JOURNAL OF VIROLOGY, Mar. 1995, p. 1661–1668 Vol. 69, No. 3
0022-538X/95/$04.00+0

The Hemagglutinin Envelope Protein of Canine Distemper Virus (CDV) Confers Cell Tropism as Illustrated by CDV and Measles Virus Complementation Analysis

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Measles virus (MV) and canine distemper virus (CDV) are morbilliviruses that cause acute illnesses and several persistent central nervous system infections in humans and in dogs, respectively. Characteristically, the cytopathic effect of these viruses is the formation of syncytia in permissive cells. In this study, a vaccinia virus expression system was used to express MV and CDV hemagglutinin (HA) and fusion (F) envelope proteins. We found that cotransfecting F and HA genes of MV or F and HA genes of CDV resulted in extensive syncytium formation in permissive cells while transfecting either F or HA alone did not. Similar experiments with heterologous pairs of proteins, CDV-F with MV-HA or MV-F with CDV-HA, caused significant cell fusion in both cases. These results indicate that in this expression system, cell fusion requires both F and HA; however, the functions of these proteins are interchangeable between the two types of morbilliviruses. Human-mouse somatic hybrids were used to determine the human chromosome conferring susceptibility to either MV and CDV. Of the 12 hybrids screened, none were sensitive to MV. Two of the hybrids containing human chromosome 19 formed syncytia following CDV infection. In addition, these two hybrids underwent cell fusion when cotransfected with CDV-F and CDV-HA (but not MV-F and MV-HA) glycoproteins by using the vaccinia virus expression system. To discover the viral component responsible for cell specificity, complementation experiments coexpressing CDV-HA with MV-F or CDV-F with MV-HA in the CDV-sensitive hybrids were performed. We found that syncytia were formed only in the presence of CDV-HA. These results support the idea that the HA protein is responsible for cell tropism. Furthermore, while the F protein is necessary for the fusion process, it is interchangeable with the F protein from other morbilliviruses.

Canine distemper virus (CDV) and measles virus (MV) are members of the genus *Morbillivirus* within the family *Paramyxoviridae*. Both CDV and MV are associated with acute illnesses and several persistent central nervous system infections, some of them fatal (17, 22, 28, 37). Moreover, a transient immunodeficiency develops during the course of the acute disease, and consequently it has been suggested that measles can serve as a model for studying immune restoration (13).

The pathogenesis of a morbillivirus infection begins with viral attachment to the surface of the host cell. In nature, CDV and MV are specific to their hosts, i.e., dogs and humans, respectively; nevertheless, in vitro both viruses can infect cell lines of human or simian origin. After attachment, fusion of the virion envelope with the cellular plasma membrane takes place (18). These processes are elicited by two virus-encoded glycoproteins of the lipid envelope. The major glycoprotein, hemagglutinin (HA), possesses hemagglutination activity and is responsible for the attachment of the virus to the host cell. The second glycoprotein, the fusion (F) protein, mediates the fusion of the cellular and viral membranes and subsequently causes syncytium formation (24, 31). For the paramyxoviruses in general, there has been some controversy about the role of HA in the fusion process. For example, it has been found by some that the F protein is sufficient to cause cell fusion (2, 14, 29), whereas others have shown that induction of cell fusion required both F and HA glycoproteins (9, 16, 25, 32, 35, 39).

In this study, a vaccinia virus transient-expression system was used to further define the significance of the HA glycoprotein in the fusion process of morbilliviruses. Different combinations of F and HA proteins from MV and CDV were coexpressed in cell lines and in somatic hybrids to determine whether functional complementation in the fusion process can take place. Our results support the idea that the HA glycoprotein plays a crucial role in determining cell specificity.

MATERIALS AND METHODS

Cells and viruses. Human epithelial cells (HeLa and HeLa T4), murine fibroblasts (LA-9), and simian kidney cells (CV-1 and Vero) were used in these experiments. Mouse-human hybrid cell lines (Table 1) (33) were a generous gift from Lester Shulman, Shiba Medical Center, Tel-Hashomer, Israel. Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 2 mM glutamine, 60 mg of penicillin per liter, and 100 mg of streptomycin per liter. The Edmonston MV strain and the Onderstepoort CDV strain were routinely grown in CV-1 cells. A vaccinia virus encoding T7 polymerase (vTF7-3) was provided by B. Moss, National Institutes of Health, Bethesda, Md. (11).

Construction of recombinant plasmids. Initially, to isolate the full-length F and HA genes from both CDV and MV, two cDNA libraries were constructed. These were screened with specific 20-mer oligonucleotides corresponding to previously reported sequences of CDV and MV F and HA genes (1, 4, 7, 30). All four cDNA clones were isolated in their entirety. For expression of proteins in the vTF7-3 system, we cloned genes into the multiple-cloning site of the vector pTM-1 (a gift from O. Elroy-Stein, Tel-Aviv University) downstream of the T7 promoter (10, 26). Four constructs were prepared by PCR technology. In each case, *Nco*I sites were introduced at the 5′ ends of the coding regions of the gene of interest, replacing the original ATG translation initiation codon. This was done by digesting the PCR products once with *Nco*I and then once at a convenient restriction site downstream of the 5′ end (keeping the PCR fragments as short as possible) and finally cloning these fragments into the remainder of the 3′ region of each gene. *Taq* polymerase was purchased from Cetus, and the reactions were carried out as specified by the manufacturers of the Hybaid Intelligent Heating Block (Hybaid, Middlesex, United Kingdom). The sequences of all the PCR portions cloned in the recombinant plasmids were confirmed, and

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TABLE 1. Ability of mouse-human hybrids to undergo cell fusion following either MV/CDV infection or viral glycoprotein coexpression

Cell line	Human chromosome(s)	Viral infection[a]		Coexpression in vv/T7 system	
		MV	CDV	MVgp[b]	CDVgp
157BnpT-4	3, 11, 14, X	−	−	−	−
AIM-15a-A1	1, 2, 11, 12, 13, 14, 15, 17, 18, 20, 21, X	−	−	−	−
AIM-15a-A2	1, 2, 11, 13, 14, 15, 17, 18, 21, X	−	−	−	−
AIM-15a-A3	1, 2, 5, 11, 13, 14, 15, 17, 18, 21, X	−	−	−	−
AIM-15a-B5	1, 2, 11, 13, 14, 15, X	−	−	−	−
AIM-15a-C2	1, 2, 7, 11, 12, 13, 14, 15, 17, 18, 21, X	−	−	−	−
AIM-15a-C3	1, 2, 7, 11, 12, 13, 14, 15, 17, 18, 20, 21, X	−	−	−	−
AIM-15a-C5	1, 2, 7, 11, 13, 14, 15, 17, 18, 20, 21, X	−	−	−	−
IL-115	7, 11, 12, 15, 17, 19	−	+	−	+
Il-115-4d	11, 12, 17, 19	−	+	−	+
L-287-C	7, 11, 17, X	−	−	−	−
WAV-A17	21	−	−	−	−

[a] Cells were infected at 1 PFU per cell.
[b] gp, glycoprotein.

the clones were further tested by in vitro coupled transcription-translation reactions (Promega TNT; technical bulletin no. 126).

(i) pTM-F-MV. Oligonucleotides a and b corresponding to the 5′ and 3′ ends of the F gene (Table 2) were used as primers in PCR. The resulting fragment was digested with *Nco*I and *Bam*HI and ligated with a pTM-1 clone containing the full-length F cDNA from which the corresponding fragment (plus regions from the polylinker) was removed. Next, the pTM-1 derivative was digested with *Bam*HI plus *Hpa*I, and a 1,500-bp fragment was removed from the gene and replaced by a corresponding *Bam*HI-*Hpa*I fragment isolated from the original F-MV cDNA clone.

(ii) pTM-HA-MV. Oligonucleotides c and d (Table 2) generated a PCR fragment which was digested with *Nco*I plus *Nhe*I and subcloned into a pTM-1 clone containing the full-length cDNA HA gene digested with the same enzymes (replacing the corresponding 5′ fragment that was removed).

(iii) pTM-F-CDV. Oligonucleotides e and f were used in PCR to form a DNA fragment which was then digested with *Nco*I plus *Eco*RI. The resulting 570-bp fragment was ligated into a *Nco*I-*Eco*RI window created in pTM-1. The clone was subsequently digested with *Eco*RI and was ligated with a portion of the 3′ end of the F-CDV gene cloned from the original cDNA.

(iv) pTM-HA-CDV. Oligonucleotides g and h were used in PCR to produce a DNA fragment that was digested with *Nco*I plus *Xho*I and cloned into the *Nco*I-*Xho*I sites of the pTM-1 polylinker. The construct was then digested with *Xho*I plus *Sal*I, and the 3′ region from the original HA-CDV gene was cloned, replacing the existing fragment.

vTF7-3 infection–pTM-1 transfection assays. In the vaccinia virus T7 expression system (vvT7), cells were first infected with vTF7-3 (a vaccinia virus encoding T7 polymerase) and then transfected with the recombinant plasmids carrying the genes of interest under the control of the T7 promoter. Confluent 35-mm dishes of cells were infected with vTF7-3 at a multiplicity of infection of 50 for 45 min and then transfected with 3 to 5 μg of DNA by using Lipofectin or Transfectase (Gibco-BRL) as specified by the manufacturer (3).

Fusion assays. Cells were cotransfected by the method described above with 3 to 5 μg each of various DNA combinations of F and HA plasmids. At 24 h postinfection, the cells were fixed in methanol, stained with Giemsa, and photographed.

RESULTS

Coexpression of F and HA proteins in CV-1 cells mediates cell fusion. To study fusion in morbilliviruses, the recombinant vvT7 expression system was used. The cDNA clones encoding the F and HA proteins of MV and CDV were cloned into the pTM-1 vector. CV-1 cells were infected with vTF7-3 and transfected with either pTM-F-MV or pTM-HA-MV. The F and HA recombinant proteins were detected by immunoprecipitation. HA is accumulated to better than 10% of the total [^{35}S]methionine incorporated during the course of an experiment. The relative amount of F protein was found to be extremely small compared with that of HA protein (data not shown). However, in both cases the amount of recombinant protein was found to be sufficient for biological activity, as illustrated in the following experiments. To establish the presence of functional recombinant proteins, the cultures were examined. Transfecting either F or HA alone under various experimental conditions (e.g., at pH 5 to 8 or in a coincubation with uninfected CV-1 cells) had no effect. On the other hand, introduction of both F and HA genes into CV-1 cells resulted in extensive syncytium formation. Similarly, syncytia were formed when F and HA genes of CDV were coexpressed in CV-1 cells (Fig. 1). A comparison of panels 2 to 6 in Fig. 1 with panels 7 and 8 illustrates that in the absence of fusion the vaccinia virus-infected cells undergo extensive contraction. This might reflect progression of two simultaneous phenomena: (i) cytopathic effect elicited by vaccinia virus alone and (ii)

TABLE 2. Oligonucleotides used to clone viral glycoproteins genes

Gene	Oligonucleotide sequence (5′-3′)	Gene location[a]	Reference
F-MV	a. GTGCCATGGGTCTCAAGGTGAACG	583–601	30
	b. TCAGAGCGACCTTACATAGG	2215–2235	
HA-MV	c. GTGCCATGGCACCACAACGAGACCGGATA	21–44	1
	d. CGATTGGTTCCATCTTCCCG	1848–1867	
F-CDV	e. CAGCCATGGGCAGGGGAATCCCCAAAAGC	86–109	4
	f. ATGCCGGATCGACCTTA	2008–2024	
HA-CDV	g. CAGCCATGGTCCCCTACCAAGACAAG	21–41	7
	h. AGTTGGCGATGTCAGCCT	1765–1782	

[a] Nucleotide number according to numeration in cited reference.

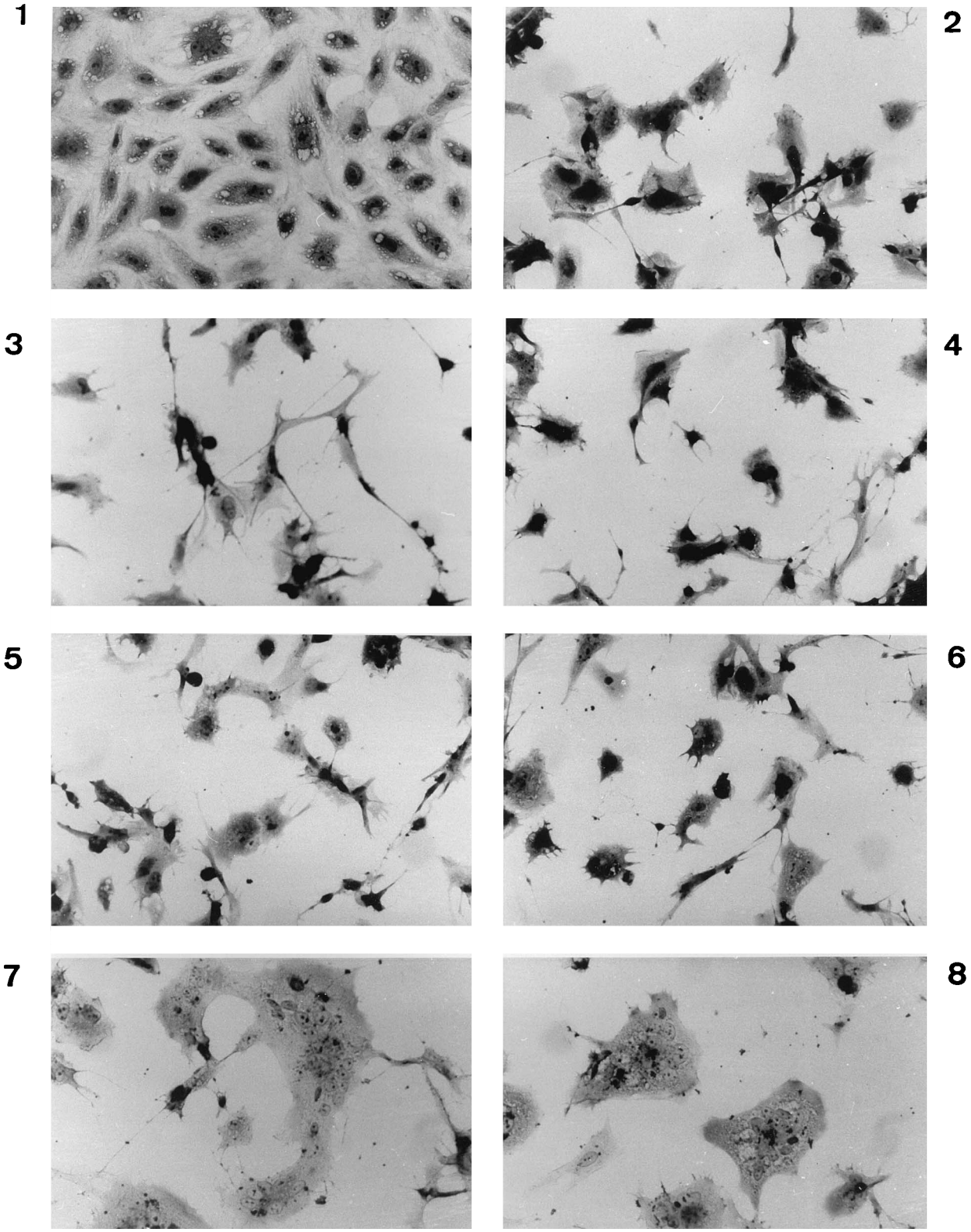

FIG. 1. Induction of fusion in CV-1 cells by coexpression of HA and F of MV and CDV. The photographs were taken at 24 h postinfection. 1, Mock-infected cells; 2, vTF7-3-infected cells; 3, vTF7-3-infected, pTM-HA-MV-transfected cells; 4, vTF7-3-infected, pTM-HA-CDV-transfected cells; 5, vTF7-3-infected, pTM-F-MV-transfected cells; 6, vTF7-3-infected, pTM-F-CDV-transfected cells; 7, vTF7-3-infected, pTM-F-MV- and pTM-HA-MV-cotransfected cells; 8, vTF7-3-infected, pTM-F-CDV- and pTM-HA-CDV-cotransfected cells. Cell fusion was observed as early as 8 h postinfection. Magnification, ×110.

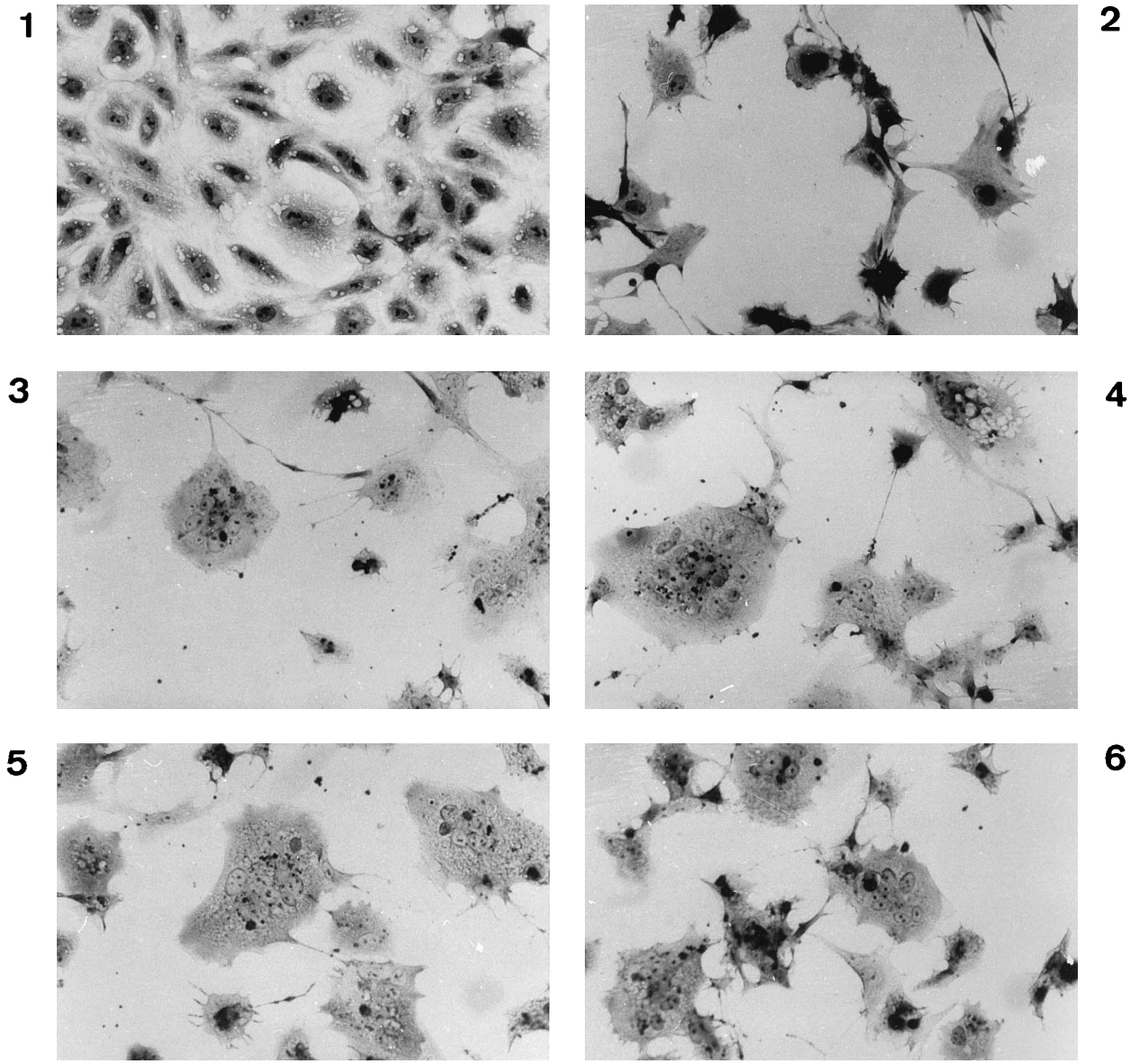

FIG. 2. Functional complementation between MV-HA and CDV-F in CV-1 cells. The photographs were taken 24 h postinfection. 1, Mock-infected CV-1 cells; 2, vTF7-3-infected CV-1 cells; 3, vTF7-3-infected, pTM-F-MV- and pTM-HA-MV-cotransfected cells; 4, vTF7-3-infected, pTM-F-CDV- and pTM-HA-CDV-cotransfected cells; 5, vTF7-3-infected, pTM-F-CDV- and pTM-HA-MV-cotransfected cells; 6, vTF7-3-infected, pTM-F-MV- and pTM-HA-CDV-cotransfected cells. Magnification, ×110.

expression of both proteins, leading to syncytium formation. Such syncytium formation could be achieved in both human and monkey cells (permissive for the viruses) but not murine cells such as LA-9.

Functional complementation between MV and CDV glycoproteins in syncytium formation. CDV and MV are closely related morbilliviruses that exhibit immunological cross-reactivity and share sequence homology, yet these viruses infect different hosts in nature and their HA proteins are not highly conserved (7, 17, 36). To determine whether the interaction between F and HA in the fusion process is virus specific, we determined if MV-HA could replace CDV-HA in syncytium formation mediated by CDV-F. Cotransfection of MV-HA and CDV-F resulted in syncytium formation in CV-1 cells (Fig. 2). Compared with the homologous systems, however, the extent and size of the heterologous F- and HA-mediated syncytia were somewhat smaller as assessed by the smaller number of nuclei per syncytium and the rate of their appearance. In a reciprocal experiment, i.e., cotransfection with CDV-HA and MV-F, considerable cell fusion in CV-1 cells was observed, although to a lesser extent than that of the CDV-F and CDV-HA pair. The results shown in Fig. 1 and 2 therefore illustrate that both F and HA glycoproteins are necessary for cell fusion and that interviral complementation is possible.

HA glycoprotein determines cell specificity in somatic hybrids. To identify the cellular component involved in virus binding, several mouse-human hybrid cell lines containing various human chromosomes were used (Table 1). These cells were tested for their ability to fuse following MV or CDV

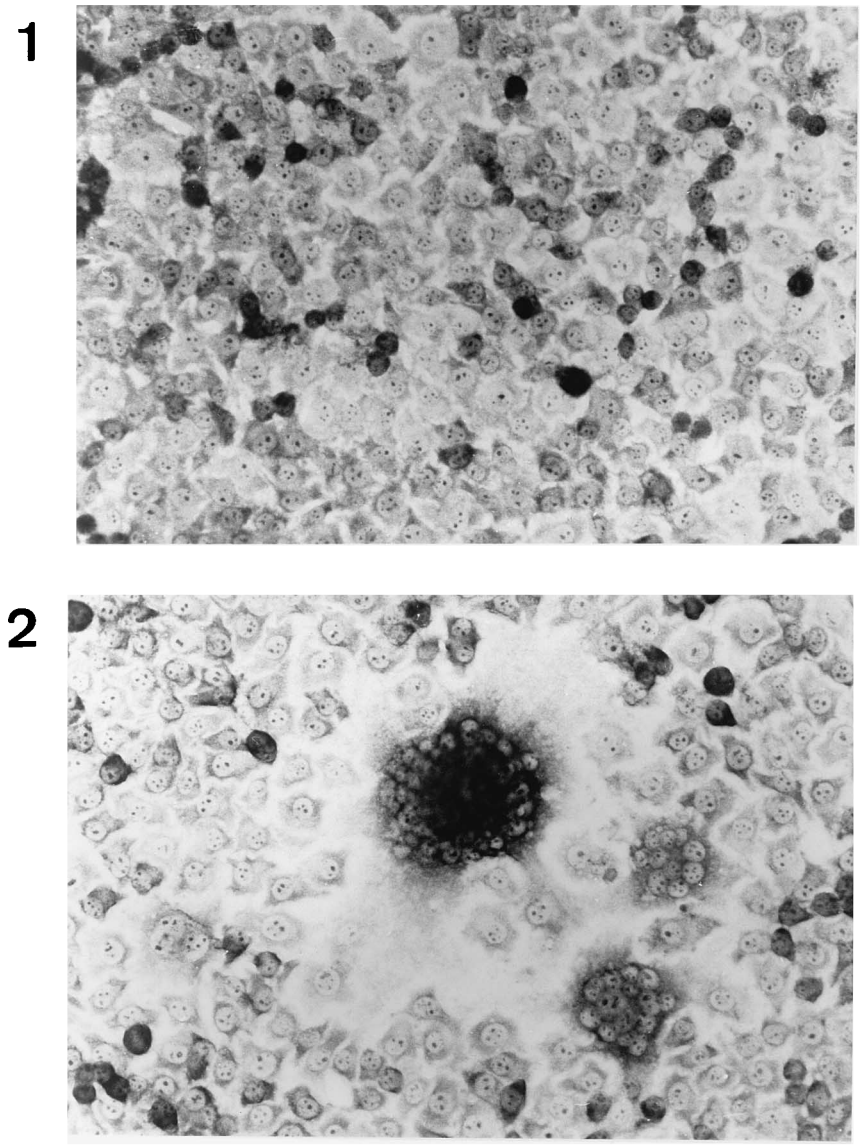

FIG. 3. MV and CDV infection of mouse-human hybrid IL-115 cells. Photographs were taken at 24 h postinfection. 1, MV-infected cells (multiplicity of infection, ~0.5). 2, CDV-infected cells (multiplicity of infection, ~0.5). Magnification, ×220.

infection. None of the somatic hybrids fused as a result of MV infection; however, following infection with CDV, two cell lines, IL-115 and IL-115-4d, formed syncytia (Fig. 3). These two cell lines are unique, because they are exclusive for human chromosome 19. This finding therefore creates a concrete distinction between the two viruses used in this study. The question arises which viral glycoprotein is responsible for the selective nature of the somatic-hybrid infection. To address this question, it was first necessary to confirm that syncytia could be elicited by using the CDV recombinant glycoproteins. Indeed, cell lines IL-115 and IL-115-4d continued to show selective susceptibility to CDV HA- and F-induced syncytium formation (Fig. 4) in contrast to their response to the MV glycoproteins.

Heterologous complementation experiments were then performed. Figure 5 depicts such an experiment, in which the IL-115 cell line was cotransfected with CDV-HA and MV-F or with CDV-F and MV-HA. Syncytia can be detected only for experiments in which the CDV-HA was expressed (Fig. 5).

These results attribute a critical role to the HA glycoprotein in determining cell tropism, while the F protein is a necessary factor in the fusion process but is interchangeable with the F protein from other morbilliviruses.

DISCUSSION

The function of the HA protein in viral attachment has been well established for various paramyxoviruses by a variety of methods (12, 15, 18, 24, 37). There is still some controversy, however, about the possible role for HA in the fusion process. Several reports have demonstrated that the F protein is sufficient for membrane fusion when recombinant viral glycoproteins are used (2, 14, 29). Alternatively, it has been shown that coexpression of the HA and F proteins was necessary (9, 16, 25,

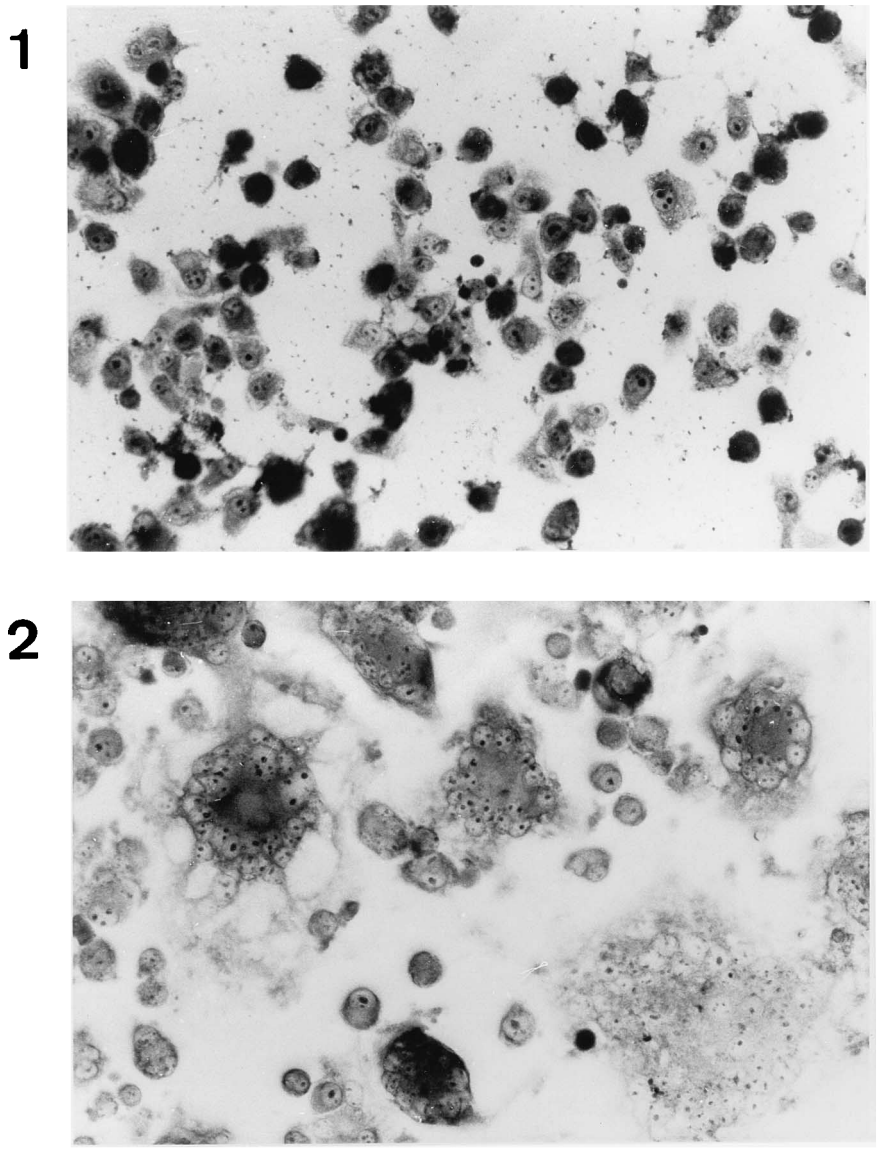

FIG. 4. Induction of fusion in mouse-human hybrid IL-115 cells by coexpression of HA and F glycoproteins. Photographs were taken at 24 h postinfection. 1, vTF7-3-infected, pTM-F-MV- and pTM-HA-MV-cotransfected cells; 2, vTF7-3-infected, pTM-F-CDV- and pTM-HA-CDV-cotransfected cells. Magnification, ×220.

32, 35, 39). Consequently, several models have been suggested, substantiating the significance of the HA protein in triggering cell fusion (18, 35). In the studies described here, using the vvT7 expression system, we observed syncytium formation only when F and HA proteins of either CDV or MV were coexpressed in permissive cells. It is generally believed that the interaction between HA and F in the fusion process is exclusively virus strain specific, and homologous pairs of F and HA from identical viruses are required to produce cell fusion (14, 16, 18, 20, 23). In one instance, reported combinations of functional F and HA proteins from different isolates of MV exhibiting a 97% amino acid identity did not form syncytia (6). By using the vvT7 expression system, functional complementation in cell fusion between the glycoproteins of far more distant viruses, namely, CDV and MV, was tested. These viruses share only a 36% amino acid sequence identity for their HA proteins and a 67% identity for their F proteins (4, 7). Nonetheless, such interspecies complementation has been illustrated.

Following the infection of mouse-human somatic hybrids with CDV, two cell lines, IL-115 and IL-115-4d, were found to be permissive for infection by CDV and permissive for syncyium formation mediated by recombinant CDV glycoproteins. The sensitivity of hybrid cells to viruses has been attributed in many cases to the presence of specific receptors encoded by the human chromosomes. The hybrid-cell analysis technique has helped to identify these specific viral receptors (8, 21, 33, 34). Therefore, noting the sensitivity of both distinct hybrid cell lines to CDV, our data suggest that the CDV receptor involved in specific recognition is encoded by human chromosome 19. Because the chromosomal content of somatic hybrid cells tends to be unstable, a Southern analysis of the hybrid cell lines was also carried out with a specific probe from the q arm of human chromosome 19. The results obtained

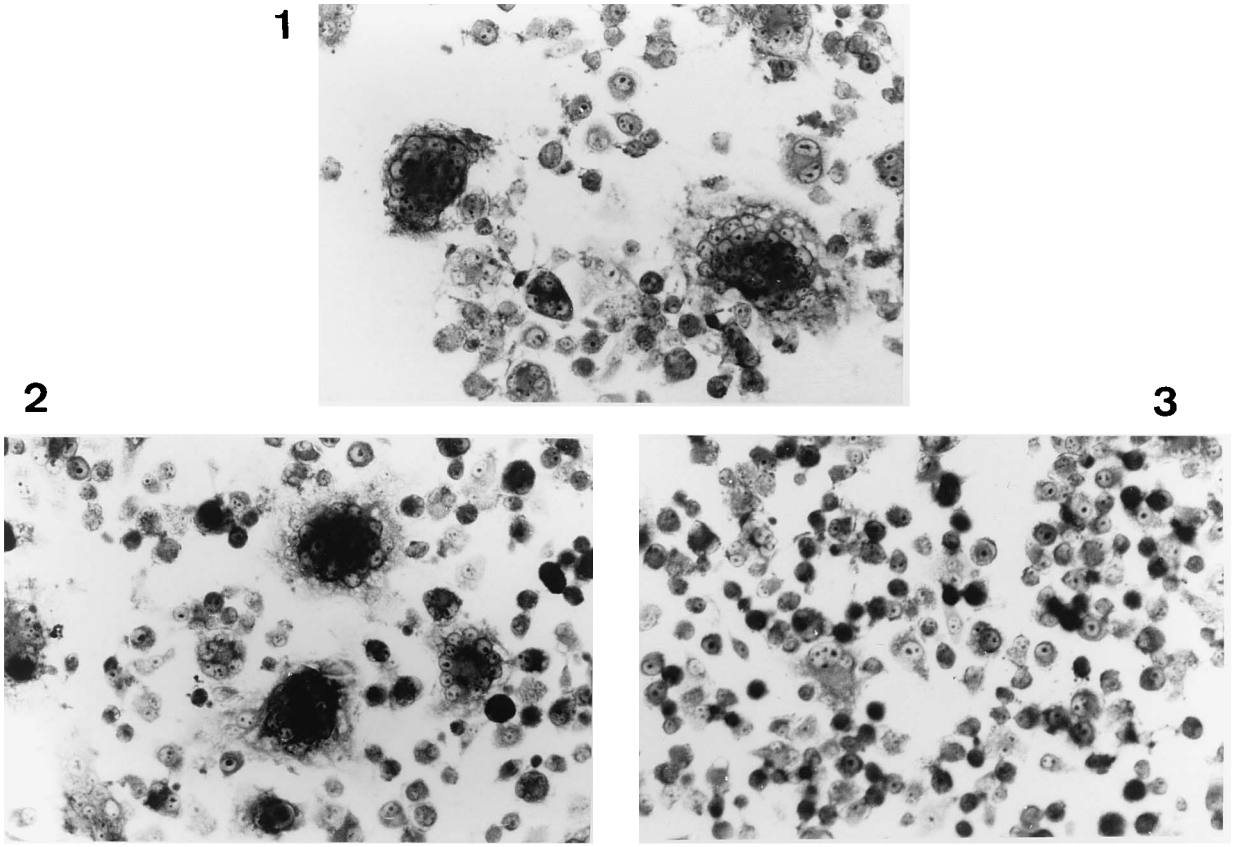

FIG. 5. Induction of fusion in mouse-human hybrid IL-115 cells with combinations of MV and CDV glycoproteins. Photographs were taken at 24 h postinfection. 1, vTF7-3-infected, pTM-F-CDV- and pTM-HA-CDV-cotransfected cells; 2, vTF7-3-infected, pTM-F-MV- and pTM-HA-CDV-cotransfected cells; 3, vTF7-3-infected, pTM-F-CDV and pTM-HA-MV-cotransfected cells. Magnification, ×125.

confirmed the presence of human chromosome 19 exclusively in the two cell lines that were identified. Because of the similarity between the two morbilliviruses CDV and MV, it may have been presumed that they share a common receptor. Recently, the receptor for MV has been identified as the human membrane cofactor protein, CD46, located on human chromosome 1 (8, 27). Naniche et al. reported that for murine fibroblasts, human CD46 is insufficient to support MV replication. Interestingly, murine B cells or CHO cells can undergo MV-dependent fusion following expression of CD46 (27). This clearly illustrates that CD46 expression alone may not be sufficient and that a requirement for an additional accessory factor may exist, as has also been suggested for human immunodeficiency virus (5, 19), ecotropic murine virus (38), and mouse hepatitis virus (40). In conclusion, the results reported here (note that IL-115 and IL-115-4d do not contain human chromosome 1 [Table 1]) illustrate a marked difference in the receptor requirements of CDV and MV.

Finally, a function for the HA of CDV was demonstrated. This was made possible by the selective permissiveness of IL-115 and IL-115-4d to MV and CDV. In the ensuing complementation experiments in which both CDV-sensitive hybrid lines were cotransfected with different combinations of glycoproteins, syncytium formation occurred only when the HA recombinant glycoprotein of CDV was used in conjunction with F protein. These results indicate that the HA protein fulfills an essential function in determining cell specificity whereas the source of the F protein is less important.

ACKNOWLEDGMENTS

We acknowledge Lester Shulman and Frank Ruddle for hybrid cell lines. We thank Bat-Sheva Bonne-Tamir for providing a probe for human chromosome 19 and Orna Elroy-Stein and Bernard Moss for gifts of vector and viruses. We also thank Bernard Moss for reviewing the manuscript.

This work was supported by the Oscar Lazar Fund for Biotechnology.

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