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Inhibition of measles virus infection and fusion with peptides corresponding to the leucine zipper region of the fusion protein

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Measles virus (MV) infections are characterized by the induction of syncytia, i.e. the fusion of infected cells. Two MV proteins, the haemagglutinin (HA) and fusion (F) proteins, are involved in this process. Synthetic peptides representing two α-helical regions of the MV F protein were studied for their ability to inhibit MV fusion. A peptide corresponding to the leucine zipper region (amino acids 455–490) inhibited MV fusion, whereas a peptide to amino acids 148–177, corresponding to the amphipathic α-helix region, did not. Fusion inhibition was also obtained with vaccinia virus-expressed HA and F, a recent wild-type MV isolate and the closely related canine distemper virus, but not with mumps virus. The F455–490 peptide did not affect the synthesis of MV F or its transport to the cell membrane. Virus–cell attachment was unaffected, but haemolysis and virus entry into the cell were inhibited. In one-step growth curves the virus yield was unaffected.

Introduction

Virus infection is initiated at the cell membrane by the attachment of the virus to a host cell receptor. The virus can then either undergo fusion at the cell membrane or be endocytosed and undergo fusion in the endosomal compartment. Paramyxoviruses and human immunodeficiency virus (HIV) use the former mechanism. The interaction of the virus with the host cell receptor probably induces a conformational reorganization, in order to potentiate the fusion protein in a biologically active form for its interaction with the host cell membrane.

Measles virus (MV), a member of the family *Paramyxoviridae*, has two glycoproteins. The initial interaction of the virus haemagglutinin (HA) with its host cell receptor CD46 triggers conformational changes in the virus fusion (F) glycoprotein (T. F. Wild *et al.*, 1994) via their specific interaction. This leads to a biologically active fusogenic form of the F protein which interacts with the host cell membrane, bringing about fusion of the virus and cell membranes.

The MV F protein contains three regions with predicted α-helical structures (Fig. 1). The first region, the fusion peptide (amino acids 116–145) at the N terminus of the F_1 protein, is hydrophobic and has been proposed to be involved in initiating virus–cell fusion. A second region, the amphipathic α-helix (AAH; amino acids 148–177), has an analogous structure in all paramyxoviruses and in certain retroviruses, but its functional role has yet to be determined. A third region, which we have previously characterized and called the leucine zipper (Buckland & Wild, 1989) extends through amino acids 455–490 and is immediately N-terminal to the transmembrane region.

In view of our original observations on MV fusion, we have re-examined the fusogenic properties of MV in the presence of synthetic peptides corresponding to these α-helical regions. We show here that a peptide (F455–490) representing the leucine zipper region inhibits virus fusion, both virus–cell and cell–cell, but a peptide (F148–177) representing the AAH was unable to do so at the concentrations used.

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Methods

■ **Viruses and cells.** Vero, HeLa and B95a cells were cultivated in Dulbecco's modified Eagle's medium (DMEM) containing 5% foetal calf serum (FCS) and antibiotics. The MV strain Hallé (Horta-Barbosa *et al.*, 1971) was cultured in Vero cells. A fresh MV isolate (Lys-1), was obtained by co-cultivation with B95-8 cells of lymphocytes from an MV patient. The virus was used at the second B95-8 cell passage. The Onderspoort strain of canine distemper virus (CDV) was grown in Vero cells. A fresh isolate of mumps virus (strain Sophie) was obtained from a throat swab from a patient and isolated on Vero cells. A vaccinia virus (VV) recombinant expressing the HA and F glycoproteins of MV Hallé (VV-HA/F) (Wild *et al.*, 1992) was grown in Vero cells. Viruses were titrated by a standard plaque assay on Vero cells.

■ **Haemagglutination and haemolysis.** Haemagglutination and haemolysis assays were performed with Vervet monkey red blood cells (MRBC) as previously described (Wild & Huppert, 1980), except that the tests for haemolysis were performed in 96-well microtitre plates.

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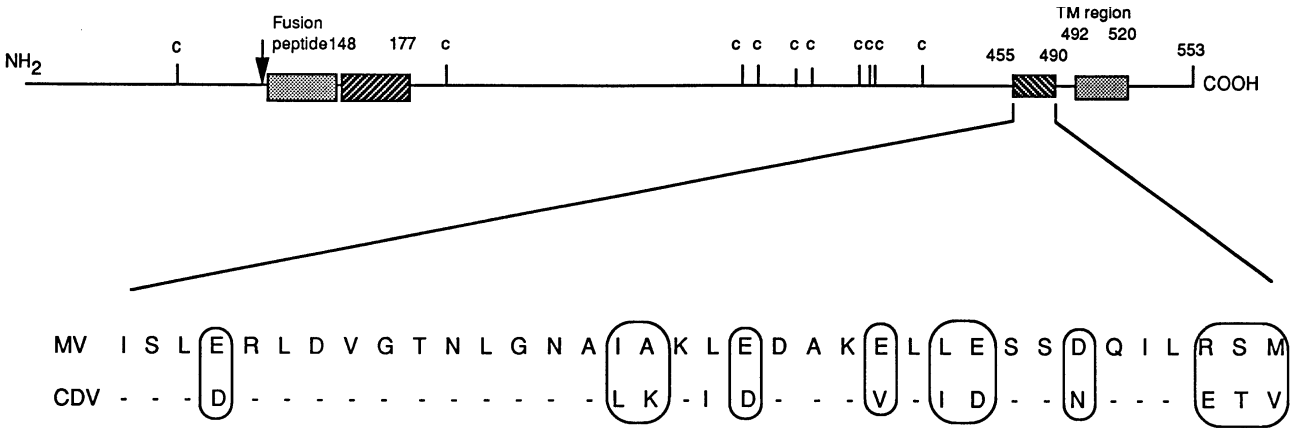

Fig. 1. Schematic representation of the MV F protein. The positions of the cysteines are indicated by 'c'. The arrow shows the cleavage site of F_0.

■ **Fusion assays.** Cells were infected with 0·1 p.f.u./cell virus for 1 h at 37 °C in 24-well Costar plates. They were then incubated in DMEM containing 2% FCS and different concentrations of peptides. The cells were scored for fusion at 24 h for VV-HA/F and at 2 days for each MV.

■ **Peptides.** Peptides were purchased from Genosys. The amino acid sequences are those from the MV Hallé strain (Buckland *et al.*, 1987).

■ **Flow cytometry.** Cells were incubated with MV anti-F(MAb 186; Malvoisin & Wild, 1990) or anti-HA (MAb 55; Giraudon & Wild, 1985) for 30 min at 4 °C (ascites fluids diluted 1:500). After washing three times in PBS the cells were incubated with a 1:100 dilution of fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse Ig (Dako) for 30 min at 4 °C. After the fourth washing in PBS, the cells were examined in a FACScan (Becton Dickinson).

Results

Inhibition of MV-induced fusion with synthetic peptides

We studied the ability of synthetic peptides representing the α-helical regions 148–177 and 455–490 to block MV-induced fusion. To investigate this phenomenon two systems were used: (i) HeLa or Vero cells infected with a VV-recombinant expressing the MV HA and F glycoproteins (VV-HA/F); (ii) MV-infected Vero or B95a cells.

In VV-HA/F-infected HeLa or Vero cells, the peptide F455–490 completely inhibited fusion at concentrations as low as 12 μM and partially down to 1·5 μM (Table 1). Similarly, in MV Hallé strain-infected Vero cells, fusion was completely inhibited at 3 μM. The phenomenon was not restricted to laboratory-adapted MV strains, as a low passage wild-type strain (Lys-1) in B95a cells was even more sensitive to F455–490 peptide inhibition. In contrast to these results, peptide F148–177 failed to inhibit fusion at concentrations as high as 40 μM (data not shown).

In our original studies, the F455–490 region was identified because it contained a structure analogous to a leucine zipper (Buckland & Wild, 1989). In order to characterize the active region, two overlapping peptides, F455–470 and F468–487, were tested for fusion inhibition. F455–470 failed to inhibit MV-induced fusion in Vero cells at concentrations up to 400 μM, whereas F468–487 inhibited fusion at 400 μM and

Table 1. MV-induced cell fusion is specifically inhibited by synthetic peptide F455–490

VV-HA/F, MV (Hallé strain), CDV or mumps virus were used to infect Vero, B95a or HeLa cells at 0·1 p.f.u./cell and then incubated with different concentrations of F455–490 peptide. Syncytium formation was recorded at 24 h for the VV-HA/F infections and at 2 days for the other viruses. 4+, ⩾ 90% of cell sheet in syncytia; 3+, 60%; 2+, 30%; +, 10%.

		Syncytia formation with indicated concentration of F455–490							
Virus	Cells	12 μM	6 μM	3 μM	1·5 μM	750 nM	375 nM	177 nM	0
VV-HA/F	Vero	−	+	2+	3+	4+	4+	4+	4+
VV-HA/F	HeLa	−	2+	3+	3+	3+	3+	3+	3+
MV Hallé	Vero	−	−	−	+	+	+	+	3+
MV Lys-1	B95a	−	−	−	−	−	2+	3+	3+
		96 μM	24 μM	12 μM	6 μM	3 μM	1·5 μM	0	
CDV	Vero	−	−	+	2+	3+	4+	4+	
Mumps virus	Vero	4+	4+	4+	4+	4+	4+	4+	

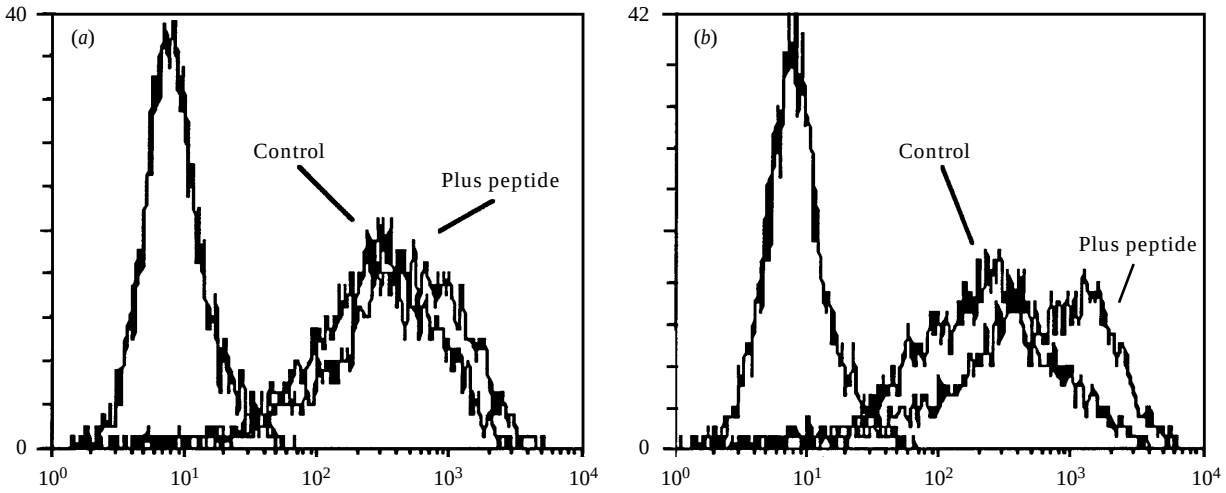

Fig. 2. Expression of MV F (*a*) and HA (*b*) proteins in the presence of F455–490 peptide. Vero cells were infected with MV and the expression at the cell surface of HA and F proteins was examined by FACScan analysis. Cells were incubated with MV anti-F MAb 186 (Malvoisin & Wild, 1990) or anti-HA MAb 55 (Giraudon & Wild, 1985) for 30 min at 4 °C (ascites fluids diluted 1 : 500). After washing three times in PBS the cells were incubated with a 1 : 100 dilution of FITC-conjugated goat anti-mouse Ig (Dako) for 30 min at 4 °C. After the fourth washing in PBS, the cells were examined in a FACScan (Becton-Dickinson).

partially at 200 μM. When the two peptides were mixed together to test for synergic effects, fusion was not inhibited at concentrations up to 100 μM.

To confirm the specificity of the fusion inhibition by F455–490, its activity was tested using the closely related CDV and the unrelated mumps virus (Table 1). Complete inhibition of CDV-induced fusion was obtained with 24 μM peptide and partial inhibition over a range of 12–3 μM. In contrast, no fusion inhibition was observed with mumps virus with peptide concentrations as high as 96 μM.

Effect of F455–490 on F expression

The F455–490 peptide may interfere with transport of the F protein or alternatively may directly interfere with the fusogenic properties of the protein at the cell surface. To study these possibilities, Vero cells were infected with MV and then incubated in the presence of 25 μM peptide. The surface expression of the HA and F proteins was studied using FACScan analysis 24 h after infection in the presence or absence of F455–490 peptide (Fig. 2). The presence of the peptide did not influence the quantity of either HA or F expressed at the cell membrane.

Virus–cell fusion

Two types of virus-induced fusion have been described: virus–cell and cell–cell. It is unknown if the same mechanism is involved in both processes. Our studies so far have described the inhibition of cell-to-cell fusion. To investigate if the F455–490 peptide affected virus cell entry, MV was titrated in a standard haemagglutination assay in the presence or absence of 50 μM peptide. The peptide did not affect the titre, so did not inhibit the attachment of the virus to MRBC. Studies of haemolysis of MRBC showed that the F peptide efficiently inhibited lysis with a 50% inhibition at 20 nM (Fig. 3). In confirmation of the previous results, the peptides F148–177 and F468–487 did not inhibit fusion even at concentrations as high as 100 μM.

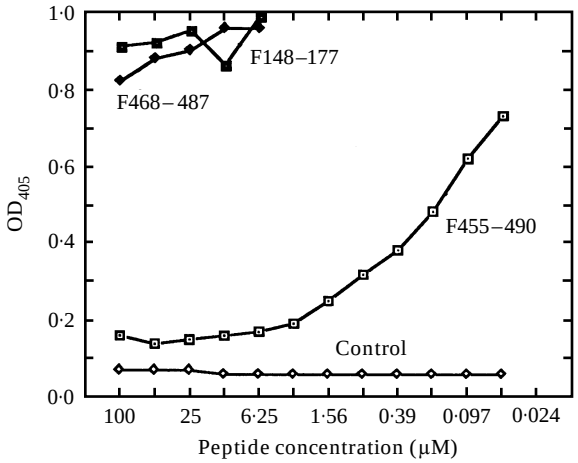

Fig. 3. Inhibition of MV-induced haemolysis. MRBCs were incubated with MV in the presence of different concentrations of synthetic peptides. The tests were performed as previously described (Wild & Huppert, 1980) except they were performed in 96-well microtitre plates.

To confirm that the F455–490 peptide could block virus–cell fusion, we tested its ability to block the entry of the virus into the cell. Vero cells were infected with MV in the presence or absence of 25 μM F455–490 peptide at 37 °C. Virus which had not entered was neutralized with an anti-HA MAb 55 and after washing, the cells were overlaid with medium containing

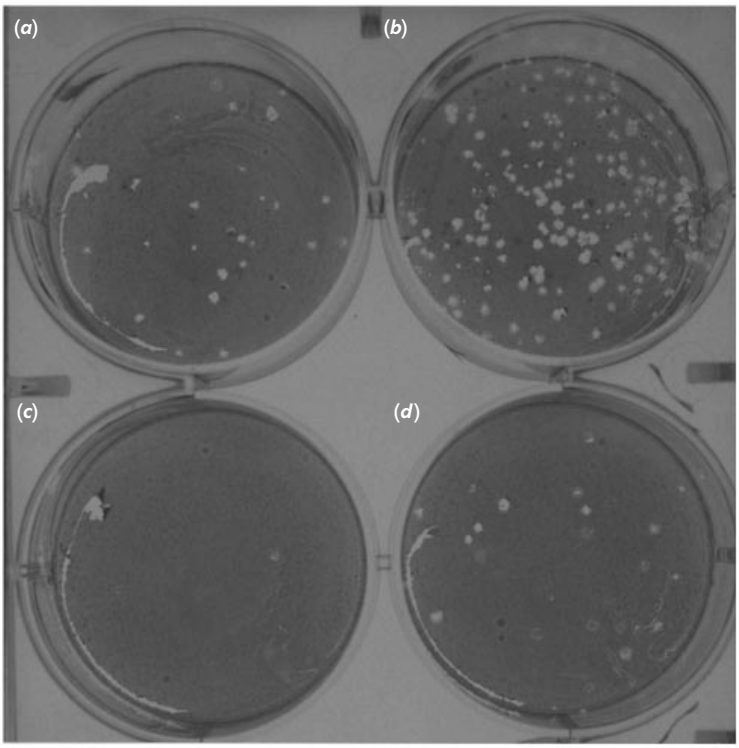

Fig. 4. Inhibition of MV infection by F455–490 peptide. Vero cells were infected with 100 µl (*a*, *b*) or 10 µl (*c*, *d*) of MV (Hallé strain) in the presence (*a*, *c*) or absence (*b*, d) of 25 µM peptide at 37 °C for 1 h. Extracellular virus was neutralized with an anti-MV HA MAb and then cells were overlaid with 1% agar.

Table 2. Effect of F455–490 peptide on MV replication

Vero cells were infected with MV (5 p.f.u./cell) and then incubated in the presence or absence of 25 µM F455–490 peptide. Virus was titrated on Vero cells.

	Virus titre (p.f.u./ml)	
	24 h	48 h
Control		
Cells	8×10^6	5×10^7
Supernatant	2×10^5	8×10^6
Plus F455–490 peptide		
Cells	7×10^6	2×10^7
Supernatant	1×10^5	2×10^6

1% agar (Fig. 4). The presence of the peptide during infection reduced the number of plaques by over 90%.

Virus release

The results presented show that the F455–490 peptide blocked both virus entry and cell-to-cell fusion. The F protein was transported and presented at the cell membrane but failed to initiate fusion in the presence of this peptide. To investigate if this state could affect the assembly of virus particles, Vero cells were infected with MV (m.o.i. 5) and then incubated in the presence of 25 µM F455–490. The virus produced in the cells and that released into the medium was titrated (Table 2). The peptide did not affect the yield of virus. In contrast, studies using a lower m.o.i. (0·04 p.f.u./cell) showed decreased yields in the presence of the peptide (data not shown). This was presumably due to inhibition of re-infection of cells during subsequent virus cycles.

Discussion

In the present study, we examined the mode of inhibition of a peptide (F455–490). At peptide concentrations that blocked fusion, the F protein was synthesized and transported to the cell surface. Virus attachment to MRBCs was unaffected, but lysis of MRBCs was inhibited with concentrations as low as 20 nM (50% inhibition). In confirmation of these results, MV plaque formation was inhibited more than 90% by F455–490. In contrast, the yield of virus under one-step growth conditions was unaffected. However, infections at low m.o.i. (0·04 p.f.u./cell) gave reduced yields (data not shown), presumably due to the inhibition of subsequent rounds of infection.

Peptides representing two different regions of the MV F protein, amino acids 116–118 (Norrby, 1971; Richardson *et al.*, 1980) and 455–490, have been shown to inhibit fusion. In both cases, virus attachment was not affected. In the case of the tripeptide analogue (116–118), it has been proposed that this prevents fusion by competing with the fusion protein for a cellular receptor (Richardson *et al.*, 1980). However, escape mutants that resist the effect of the tripeptide were found to possess mutations in the cysteine-rich region of MV F (Hull *et al.*, 1987), which we have shown to be the site of interaction with the MV HA (T. F. Wild *et al.*, 1994). This suggests that

rather than having a direct effect on fusion, the tripeptide disrupts the F-HA interaction known to be a prerequisite for MV fusion (T. F. Wild *et al.*, 1994). In contrast, peptide F455–490 probably has a direct effect on MV fusion. Recently, Lambert *et al.* (1996) studied the fusion inhibition of overlapping 35-mer peptides for the leucine-rich region of MV. Anti-fusion activity was still observed with peptides starting nine amino acids N-terminal to our studies. This may imply that the initial interaction takes place within a larger region.

Results we obtained with CDV-MV F chimeras (T. F. Wild *et al.*, 1994) suggest that the MV F leucine zipper could also play a role in the native non-fusogenic MV F, forming a coiled-coil structure with the AAH, thereby temporarily restraining and masking the fusion peptide. However, the demonstration that mutation of the leucines in the zipper abrogates fusion (but not oligomerization of the MV F protein), leads us to propose that the MV leucine zipper also plays a role in the formation of the fusion pore (Buckland *et al.*, 1992). If the MV F leucine zipper also functions in the recruitment of MV F molecules into the fusion pore (Buckland *et al.*, 1992), the mode of action of the F455–490 peptide could be to inhibit competitively this process.

In conclusion, the interpretation of our results together with those of others (Matthews *et al.*, 1994; C. T. Wild *et al.*, 1994) suggests that there is much in common between the mechanism of virus fusion used by paramyxoviruses and HIV.

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