A RhoA-derived peptide inhibits syncytium formation induced by respiratory syncytial virus and parainfluenza virus type 3

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The fusion glycoproteins of human respiratory syncytial virus (RSV) and human parainfluenza virus type-3 (PIV-3) mediate virus entry and syncytium formation. Interaction between the fusion protein of RSV and RhoA, a small GTPase, facilitates virus-induced syncytium formation. We show here a RhoA-derived peptide inhibits RSV and syncytium formation induced by RSV and PIV-3, both in vitro by inhibition of cell-to-cell fusion and in vivo by reduction of peak titer by 2 \( \log_{10} \) in RSV-infected mice. These findings indicate that the interaction between these two paramyxovirus fusion proteins and RhoA is an important target for new antiviral strategies.

Human respiratory syncytial virus (RSV) and parainfluenza virus type-3 (PIV-3) belong to the Paramyxoviridae family. RSV and PIV-3 are the main viral causes of acute lower respiratory tract illness in infants. There is no effective antiviral therapy or vaccine available for either virus. RSV and PIV-3 each have two main surface envelope glycoproteins: an attachment protein (RSV G or PIV-3 hemagglutinin-neuraminidase) and a fusion protein (F). The G or hemagglutinin-neuraminidase glycoproteins are thought to mediate virus attachment to the cell receptor. The receptor for PIV-3 is sialic acid, and RSV G may bind to heparan sulfate. The F glycoprotein mediates at least two essential steps in the virus life cycle that require membrane fusion: it promotes fusion of the viral and cellular membranes with subsequent transfer of viral genome material into the cell, and promotes fusion of the infected cell membrane with those of adjacent cell membranes, leading to syncytia formation. The F glycoproteins of RSV and PIV-3 are synthesized as inactive precursors, which are co-translationally modified by the addition of N-linked glycosylation in the endoplasmic reticulum. The F precursor is cleaved by cellular trypsin-like endoproteases into two disulfide-linked subunits, F1 and F2, before reaching the cell surface, and is assembled as a higher-order homooligomer. RSV can infect cells in vitro despite lacking the G glycoprotein, indicating that F may have additional functions of attachment to a host coreceptor.

The process of membrane fusion is essential for the entry of both cell-free and cell-associated virus. RSV and PIV-3 share the properties of syncytium formation and pH-independent fusion from without. Common host determinants may be involved in the essential processes required for viral entry and syncytium formation. The fusion glycoproteins of these viruses share elements of similar function, structure and, in some cases, sequence, indicating a common membrane fusion mechanism. In particular, paramyxovirus F proteins share conserved hydrophobic sequences at their amino termini, important for interactions with the lipid bilayer, followed by a region of heptad repeats.

Although much is known about the properties of membrane fusion in selected cellular compartments or in artificial lipid membranes, the molecular mechanisms for both virus-induced and cellular fusion reactions remain mostly undefined. Cell-to-cell fusion mediated by some viral envelope proteins involves cellular actin cytoskeleton and cell surface integrins. Host cellular proteins that maintain cell membrane integrity and cell mobility, such as RhoA, might be expected to be involved in virus-induced fusion and syncytium formation; however, there is no direct evidence at present for their involvement.

RhoA binds to RSV F protein and mediates virus-induced syncytium formation. In addition, RhoA amino acids 67–110 bind to RSV F amino acids 146–155. RhoA, a small GTPase of the Ras superfamily, controls a plethora of biological functions including actin reorganization, gene expression, cell morphology, cell motility and cell proliferation. We report here a RhoA peptide derived from the domain that binds the RSV F protein has potent antiviral activity against RSV and PIV-3 infection in cell culture and in RSV-infected mice. These data provide additional evidence for conserved structural and functional features between the fusion proteins of RSV and PIV-3 and for the possibility of common mechanisms involving virus-induced membrane fusion. The inhibitory effect of this RhoA \( \alpha_{77-95} \) peptide may be useful for the development of new antiviral strategies with broad application.

Effects of RhoA peptides on RSV-induced syncytium

We synthesized three overlapping 19-amino-acid peptides spanning the F-binding domain of RhoA (amino acids 67–110) and used these to study the effect of peptides on virus-induced syncytium formation. Pretreatment of virus inoculum with the RhoA\( \alpha_{77-95} \) peptide completely blocked plaque formation, whereas pretreatment with RhoA\( \alpha_{67-85} \) did not have any effect on the plaque formation relative to media-treated controls (Fig. 1). The RhoA\( \alpha_{77-95} \) peptide did not block RSV-induced syncytium forma-
Fig. 1 RhoA-derived peptide inhibits RSV infection and syncytium formation. Addition of RhoA
77-95 (left) but not RhoA87-105 (right) to RSV stock18 pre-
vented plaque formation in HEp-2 cells.

tion. Varying the concentration of RhoA77-95 showed that the
concentration required to inhibit number of plaques by 50%
(IC50) was 0.54 µg/ml, or 0.25 µM. Immunoperoxidase staining
did not show any RSV antigen-positive cells treated with RhoA77-
95, indicating that RSV replication was inhibited at a step before
viral protein synthesis. These data indicate that the RhoA77-95
peptide inhibits RSV at an early step of the replication cycle.

We next determined whether the RhoA77-95 peptide could in-
hibit cell-to-cell spread of RSV after infection. We used a recombi-
nant RSV (rgRSV) expressing a gene for green fluorescent protein,
located at the first position in RSV gene order. We added 5 µg/ml
RhoA77-95 to cells 0, 4 and 24 hours after RSV adsorption, and
evaluated HEp-2 monolayers by fluorescent microscopy 48 hours
after infection (Fig. 2). There was inhibition of cell-to-cell spread
and syncytia formation in rgRSV-infected cells treated with the
RhoA77-95 peptide compared with that of untreated, infected cells.
The number of infected cells increased between 4 and 24 hours
after infection, indicating the completion of one round of viral
replication, production of new virions and infection of new cells
before 24 hours. Therefore, the addition of RhoA77-95 seems to
prevent subsequent spread of cell-free virus to other cells as well as
preventing syncytium formation. These data also support the
possibility of a block at an early step in the viral life cycle.

Next, we tested whether RhoA77-95 peptide inhibition of RSV
was reversible. We dialyzed RSV–RhoA77-95 Peptide, RSV–RhoA87-105
peptide and RSV–media suspensions overnight and added these
to HEp-2 cell monolayers. There were no RSV plaques in wells
treated with dialyzed RSV–RhoA77-95 peptide suspension, in con-
trast to the presence of many plaques in wells treated with
dialyzed RSV–RhoA87-105 peptide or RSV–media suspensions (data
not shown). This indicates that the RhoA77-95 peptide binds with
high avidity to RSV F.

Cell-to-cell fusion assay
To determine whether the RhoA77-95 and RhoA87-105 peptides have
biological effects in cell-to-cell fusion induced by RSV F, we used an
assay based on the cytoplasmic activation of the reporter
gene β-galactosidase. The RSV envelope proteins F, G and
SH have been shown in this assay to optimize cell-to-cell fusi-
on16,17. We did this assay as described18 with some modifica-
tions. One cell population was infected with the recombinant
vaccinia virus vTF7-3 and transfected with three separate plas-
mids containing RSV F, G and SH genes. The other cell popula-
ion was infected with a recombinant vaccinia virus expressing
β-galactosidase. The two cell populations were mixed and either
treated with 5 µg/ml peptides or left untreated. Cell fusion was
measured by an in situ assay using X-gal staining of cells (Fig.
3a–c) or a quantitative colorimetric lysate assay for β-galactosi-
dase (Fig. 3d). Wells treated with the RhoA77-95 peptide showed
no blue fused cells (Fig. 3b), in contrast to wells treated with
the RhoA87-105 peptide (Fig. 3c) or untreated wells (Fig. 3a). The in situ
assay correlated with the quantitative colorimetric assay for the
treated and untreated wells (Fig. 3d). Control wells containing
one population of cells expressing G and SH and another popu-
lation of cells infected with vaccinia virus expressing β-galactosi-
dase did not show β-galactosidase activity (data not shown).

These data show that the cell-to-cell fusion induced by RSV F
was blocked by RhoA77-95 peptide, and indicate that the cell-to-
cell spread of virus through syncytium formation may require
the binding of F to RhoA.

RhoA77-95 peptide inhibits RhoA–RSV F interaction
To determine whether the RhoA77-95 peptide can interfere with
the interaction between F and RhoA, we did a competitive en-
zyme-linked immunosorbent assay (ELISA) (Fig. 4). We coated
wells with 25 ng purified F, and after blocking, added 50 ng
RhoA and increasing concentrations of peptide (range, 100
ng/ml to 500 µg/ml), then detected bound RhoA with mono-
clonal antibody against RhoA. We used RhoA without the pep-
tide as a positive control. As a negative control, we used Rac1
protein and RhoA87-105 instead of RhoA and RhoA77-95, respec-
tively. There was a correlation between concentration of RhoA77-95
peptide and inhibition of RhoA interaction with F. The results
for all concentrations of RhoA87-105 were similar to data from the
untreated positive control, whereas the results for Rac1 binding
to F were similar to background values. These data indicate that
the biologic effects demonstrated with the peptide in cell culture
and in vivo are based on its inhibition of the interaction between
RhoA and the viral fusion protein.

Fig. 2 Effect of RhoA77-95 added after rgRSV infection. Syncytium forma-
tion was assessed 48 h after infection. a and b, Peptide added 0 h after in-
fection. b, Phase contrast image of a. c and d, Peptide added 4 h after
infection. d, Phase contrast image of c. e, Peptide added 24 h after infe-
cession. f, No peptide added.
Fig. 3 Effect of RhoA-derived peptides on cell-to-cell fusion. HEp-2 cells expressing RSV F, G, and SH glycoproteins were untreated (a) or treated with RhoA77-95 (b) or RhoA87-105 (c), then mixed with another cell population infected with recombinant vaccinia virus expressing β-galactosidase, then assessed by in situ X-gal staining (a, b and c) or quantitative colorimetric lysate assay (d). Equal volumes of cell lysates and 2× substrate solution were mixed and the rates of substrate hydrolysis was monitored by measuring absorbance at 590 nm with a spectrophotometer. The data are presented as β-galactosidase (ng/well) produced by the cells. Each point represents an average of three experiments ± standard deviation.

Next, we determined whether the binding of RhoA77-95 peptide to F was reversible or nonreversible in the ELISA. We added RhoA protein 1 hour after allowing RhoA77-95 peptide to bind F protein, incubated this for 1 hour, and detected RhoA using monoclonal antibodies against RhoA. There was no binding of RhoA to F (data not shown). Thus, the RhoA77-95 peptide binds F with high avidity.

RhoA77-95 peptide can block infection in an animal model for RSV

To determine whether the RhoA peptide would alter the course of infection in vivo, we infected BALB/c mice intranasally with 0.1 ml containing 107 plaque-forming units (PFU) live RSV, using a well-established model. We administered 500 µg RhoA77-95 peptide, RhoA87-105 peptide or PBS intranasally immediately before or 2 hours or 4 days after RSV infection. Mice treated with the RhoA77-95 peptide immediately before or 2 hours after virus infection had no discernable illness or weight loss, whereas PBS-treated control mice experienced a typical illness pattern (Fig. 5a) with a 22% peak weight loss from baseline. Mice treated with the peptide 4 days after virus infection had no discernable illness or weight loss. PBS-treated control mice experienced a typical illness pattern on day 4 after RSV challenge showed that peptide treatment immediately before or 2 hours after virus infection reduced RSV titers by 2 log10 compared to the RSV titers in lungs from control PBS-treated mice treated with PBS or the RhoA77-95 peptide (Fig. 5b). These data indicate that the diminished illness in mice treated with the RhoA77-95 peptide before RSV infection could be due to inhibition of virus replication by the peptide.

RhoA77-95 peptide can inhibit infection by PIV-3

As the amino terminus of the RSV fusion glycoprotein is similar in structure and hydrophobicity to that of other enveloped viruses, we next determined whether the RhoA77-95 peptide could block infection with PIV-3, influenza A virus (H3N2 A/Beijing/1996), herpes simplex virus type I, vaccinia virus or a coronavirus (mouse hepatitis virus strain A59). Syncytium formation was inhibited in PIV-3-infected HEp-2 cells (Fig. 6) by the RhoA77-95 peptide. However, infection with influenza virus, herpes simplex virus type I, vaccinia virus, or coronavirus was not inhibited by the RhoA77-95 peptide (data not shown). Treatment of PIV-3 with the RhoA87-105 peptide had no effect on syncytium formation (Fig. 6). These data indicate that a common mechanism of virus-induced syncytium formation involving RhoA may be shared between these two paramyxoviruses. They also indicate that although a common motif is present at the amino terminus in the fusion proteins of many enveloped viruses, host cell components involved in syncytium formation may differ between virus families.

Discussion

The hallmark of the RSV and PIV-3 cytopathic effect in cell culture is extensive syncytium formation. The fusion glycoprotein (F) mediates virus-induced fusion at neutral pH and for PIV-3, membrane fusion depends on the density of the fusion protein on the infected cell surface. Cellular proteins contributing to paramyxovirus-induced syncytia formation have not been identified before. However, the lipid composition and other properties of the target cell membrane can influence the ability of a virus to produce syncytia. A host protein, RhoA, has been shown to bind to viral fusion glycoprotein and facilitates syncytium formation. We have shown here the inhibition of RSV- and PIV-3-induced syncytium formation by a RhoA-derived peptide (RhoA77-95) from the RSV F binding domain. This inhibition indicates that two viruses from the Paramyxoviridae family may use the same host cell protein and same binding site on this protein in the process of inducing cell-to-cell fusion, strengthening the concept of conserved functional and structural features of paramyxovirus fusion proteins. The role of RhoA in virus-induced fusion is not yet defined and might include a direct effect, or an indirect effect through signaling events on cellular cytoskeletal organization, cell shape or cell motility. The F protein interaction with RhoA might also be involved in other aspects
of virus replication including assembly, filament formation or budding.

Peptides derived from the heptad repeat region of viral fusion protein inhibit syncytium formation by homologous virus by binding to the heptad repeats interfering with fusion protein structure. The inhibitory peptide described here is of a different nature. We have shown here that a peptide derived from a cellular protein that does not have sequence homology to the viral fusion proteins can specifically inhibit virus-induced syncytium formation and possibly virus entry. In a yeast two-hybrid assay, RhoA was shown to not bind a RSV F construct expressing the terminal heptad repeat of gp41, in HIV-1 treatment similar to that of T-20, a peptide derived from the C-terminal heptad repeat of gp41, in HIV-1 treatment. The high avidity binding of the RhoA 77-95 peptide to F supports prior analysis of the RSV F–RhoA interaction by biomolecular interaction analysis based on surface plasmon resonance. This observation was also supported by data obtained from the dialysis experiment, in which the RhoA 77-95 peptide irreversibly prevents RSV-induced syncytium formation despite overnight dialysis.

Blockade of RSV and PIV-3 syncytium formation by the same peptide, RhoA 77-95 (Figs. 1 and 6, respectively), indicates that the mechanism of virus-induced membrane fusion is similar in both viruses. This provides additional evidence for conserved structural and functional features between the fusion proteins of RSV and PIV-3 indicated by sequence analysis, and raises the possibility of designing a single antiviral agent against both these paramyxovirus-induced diseases. For example, a RhoA-derived peptide (RhoA 77-95), a related peptidomimetic compound or a small molecule capable of interfering with the RhoA-fusion protein interaction could potentially have a clinical effect in RSV treatment similar to that of T-20, a peptide derived from the C-terminal heptad repeat of gp41, in HIV-1 treatment. The inhibitory effect of RhoA 77-95 in vivo on virus replication and potential for diminishing illness in the mouse model of RSV (Fig. 5) indicate that early treatment with a RhoA-derived peptide has therapeutic potential in RSV or PIV-3 infections, particularly in giant cell pneumonia, seen in patients with bone marrow trans-
plantation, lung transplantation or severe combined immunodeficiency. Although the peptide treatment immediately before or 2 hours after RSV infection prevented illness in mice, treatment on day 4 after RSV infection had no effect on the illness (data not shown). This is consistent with other antiviral approaches and reflects the fact that when illness is mediated by the T-cell response and is not direct virus-induced cytopathology, antiviral therapy must be given early or combined with immunomodulators.

In conclusion, a RhoA-derived peptide (RhoA 77-95) was shown here to inhibit RSV and PIV-3 infection and syncytium formation. RhoA 77-95 also blocked cell-to-cell-fusion in a virus fusion glycoprotein (F)-induced cell-to-cell fusion assay. Given immediately before infection or 2 hours after RSV infection, the RhoA 77-95 peptide reduced RSV titer and prevented illness in mice. Exploiting the interaction between RhoA and F protein may also be useful for designing peptidomimetic or low-molecular-weight antiviral drugs.

Methods

Virus and cells. The A2 strain of RSV was provided by R. Chanock (National Institutes of Health, Bethesda, Maryland). The rgRSV was provided by M. Peeples (Rush Medical College, Chicago, Illinois) and P. Collins (National Institutes of Health, Bethesda, Maryland). Wild-type parainfluenza virus type 3 (PIV-3), influenza A virus (H3N2 A/Beijing/1996), herpes simplex virus type I and the coronavirus, mouse hepatitis virus strain A59 strains were provided by B. Murphy, P. Wright, P. Spearman and M. Denison, respectively. RSV stocks were prepared as described.

RhoA-derived peptides. The peptides were synthesized by Research Genetics (Huntsville, Alabama) as the free acid, at approximately 70% purity in the desalted, lyophilized form. The major species in each preparation had the calculated molecular weight of the desired peptide, based on mass spectrometry. The amino-acid sequence of the peptides are: RhoA 67-85, DRL-RPLSYSPTDDVILMCF; RhoA 77-95, TDVLMCFSIDSDPSL; RhoA 87-105, DSDPSLENIPKWPTEPKH; and RhoA 77-95, Scrubbed, DDMVSIEICTSLPFDIN. All the peptides were resuspended in deionized distilled water.

Blockade of RSV infection in cell culture with peptides. For the assay, the peptides were incubated for 1 h on ice with 1 x 10^6 PFU/ml RSV at peptide concentrations of 5 and 10 µg/ml of media, then 100 µl of the suspension was added to HEp-2 cells in 96-well plates. For defining the IC50 concentrations of peptide ranging from 0.1 µg/ml to 5 µg/ml were used. After 3 d, plates were fixed with methanol and RSV-specific immunoperoxidase staining was done.

Cell fusion assay using vaccinia virus-based expression of RSV envelope glycoproteins. The ability of the RhoA 77-95 peptide to inhibit RSV F-induced cell-to-cell fusion was assessed using a quantitative assay based on the cytoplasmic activation of reporter gene β-galactosidase. One population of HEp-2 cells was infected with recombinant vaccinia virus vTF7-3, which encodes T7 polymerase, at a multiplicity of infection of 10 PFU per cell, and was transfected with plasmids encoding RSV glycoproteins F, G and SH under control of the T7 promoter (gifts from P. Collins, National Institutes of Health, Bethesda, Maryland) using LipofectAMINE (Life Technologies). At 5 h after transfection, the cells expressing viral envelope proteins were trypsinized, suspended in MEM containing 2.5% FBS to a density of 2 x 10^4 cells/ml, and incubated overnight at 32 °C. The cells were then washed and suspended in Opti-MEM (Life Technologies) at a concentration of 1 x 10^5 cells/ml. A second population of HEp-2 cells was infected with recombinant vaccinia virus expressing β-galactosidase under control of the T7 promoter (provided by E.A. Berger, National Institutes of Health, Bethesda, Maryland). At 5 h after infection, cells were trypsinized and finally suspended at a concentration of 1 x 10^5 cells/ml. The cell population expressing the viral glycoproteins was treated with 5 µg/ml RhoA 77-95 or RhoA 87-105 peptide or left untreated, and incubated for 30 min at 37 °C. The two cell populations were mixed in triplicate by adding 100 µl of each cell population to 96-well tissue culture plates, which were then incubated at 37 °C for 4 h. Cell fusion was measured by the quantitative colorimetric lysis assay for β-galactosidase or an in situ assay using X-Gal (5-bromo-4-chloro-3-indolyl β-D-galactopyranoside; Life Technologies) staining of cells. In the colorimetric lysis assay, 5 µl 20% (volume/volume) Nonidet-P40 was added to each well, and the contents were mixed by pipetting. β-galactosidase activity was quantified at ambient temperature in 96-well flat-bottomed plates by mixing 50 µl of each lysis with 50 µl 2X substrate solution (16 mM CPBG (chlorophenol-red-β-D-galactopyranoside; Boehringer), 0.12 M NaH2PO4·7H2O, 0.08 M Na2HPO4·H2O, 0.02 M KCl, 0.002 M MgSO4·7H2O and 0.01 M β-mercaptoethanol). The rate of substrate hydrolysis at ambient temperature was monitored by measuring the absorbance at 590 nm with a spectrophotometer. The quantity of β-galactosidase was calculated by comparing the hydrolysis rates for each sample with that obtained for a standard commercial preparation of Escherichia coli β-galactosidase (600 U/mg; Boehringer). β-galactosidase levels were expressed as nanograms per well. For the in situ assay, 20 µl 10X fixative solution (20% formaldehyde and 2% glutaraldehyde in PBS) was added to each well. The plates were incubated at 4 °C for 5 min. Without disturbing the settled cells, 0.15 ml medium was gently removed and replaced with 0.15 ml of 37 °C-equilibrated staining solution (5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 2 mM magnesium chloride and 1 mg/ml X-Gal). The plates were incubated overnight at 37 °C to allow complete staining. Blue-stained syncytia were viewed with an inverted phase-contrast microscope.

Competitive ELISA. Immunoaffinity-purified RSV F protein (a gift from Wyeth-Lederle–Praxis Biologicals, West Henrietta, New York) was diluted to a concentration of 250 ng/ml in carbonate/bicarbonate buffer, pH 9.6. A suspension (100 µl) containing 25 ng F protein was applied to wells of 96-well plates (Nunc, Roskilde, Denmark). Blocking was accomplished overnight with 3% nonfat dry milk and 3% BSA. Blocking buffer (100 µl) containing 50 ng RhoA or Rac1, another Rho family GT-Pase (CalBiochem, La Jolla, California), and increasing concentrations of either RhoA 77-95 or RhoA 87-105 peptide (range, 100 ng/ml to 500 µg/ml) was added separately and incubated overnight at 4 °C. RhoA added without the peptide was used as a positive control. As a negative control, Rac1 protein and RhoA 87-105 were used instead of RhoA and RhoA 77-95, respectively.

Monoclonal antibodies against RhoA or Rac1 (1:4,000 dilution; Santa Cruz Biotech, Santa Cruz, California) in blocking buffer were added after wells were washed with PBS and 0.1% Tween 20. After 1 h, plates were washed and goat antibody against mouse IgG conjugated to horseradish peroxidase (1:7,000 dilution) was added. After washing, the substrate 3,3′,5,5′-tetramethylbenzidine (Sigma) was added and the absorbance was measured at 450 nm using a ‘microtiter plate reader’ (Dynatech, Chantilly, Virginia).

Treatment of RhoA 77-95 peptide in RSV-infected mice. Pathogen-free, 8-week-old BALB/c mice were obtained from Charles River Laboratories (Raleigh, North Carolina) and were housed in a barrier facility. The mice were anesthetized and intranasally infected with 0.1 ml containing 1 x 10^4 PFU live RSV, as described. Immediately before or 2 h or 4 h after RSV infection, 500 µg RhoA 77-95 peptide or PBS were given intranasally. Lungs were obtained from five mice from each group on day 4 after infection from RSV-infected mice treated with peptide or PBS immediately before or 2 h after...
RSV infection. Five RSV-infected mice from each group were weighed for 12 d after infection. Illness was graded daily by an observer ‘blinded’ to treatment status of mice; clinical features of illness were scored as: 0, no apparent illness; 1, slightly ruffled fur; 2, ruffled fur, but active; 3, ruffled fur and inactive; 4, ruffled, inactive, hunched posture and gaunt; 5, dead.

RSV plaque assay from lung tissue. Four days after RSV infection, mice were killed by CO₂ narcosis and cervical dislocation. The lungs were removed, placed in EMEM with 10% FBS, and were quickly frozen in a bath of alcohol–dry ice. RSV titers in the lungs were measured by standard plaque assays using HEP-2 monolayers that were 80% confluent. Lungs were thawed quickly and ground with a mortar and pestle. Serial 10-fold dilutions of lung supernatants were used to infect the monolayers in triplicate, and cultures were grown under 0.75% methylcellulose in EMEM with 10% FBS. Cells were formalin-fixed 5 d after being infected, and were stained with hematoxylin and eosin; plaques were counted using a dissecting microscope. Data are presented as the geometric mean log₃ PFU per gram of lung tissue ± standard deviation (s.d.) at the dilution producing more than five plaques per well.

Blockade of PIV-3 infection in cell culture with peptides. Peptides RhO₅₃₂₃₅ or RhO₅₂₂₃₅ at concentrations of 50 μg/ml were incubated with 100 μl PIV-3 stock for 1 h on ice, then the suspension was added to HEP-2 cells in 12-well plates. PIV-3 plaques were viewed by phase contrast microscopy.

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