The viral protein corona directs viral pathogenesis and amyloid aggregation

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Artificial nanoparticles accumulate a protein corona layer in biological fluids, which significantly influences their bioactivity. As nanosized obligate intracellular parasites, viruses share many biophysical properties with artificial nanoparticles in extracellular environments and here we show that respiratory syncytial virus (RSV) and herpes simplex virus type 1 (HSV-1) accumulate a rich and distinctive protein corona in different biological fluids. Moreover, we show that corona pre-coating differentially affects viral infectivity and immune cell activation. In addition, we demonstrate that viruses bind amyloidogenic peptides in their corona and catalyze amyloid formation via surface-assisted heterogeneous nucleation. Importantly, we show that HSV-1 catalyzes the aggregation of the amyloid β-peptide (Aβ42), a major constituent of amyloid plaques in Alzheimer’s disease, in vitro and in animal models. Our results highlight the viral protein corona as an acquired structural layer that is critical for viral-host interactions and illustrate a mechanistic convergence between viral and amyloid pathologies.
The term “protein corona” refers to the layer of proteins that adhere to the surfaces of nanostructures when they encounter biological fluids. Nanoparticles adsorb biomolecules in biological fluids due to the high free energy of their surfaces. The importance of the corona layer stems from the fact that it constitutes the actual surface of interaction with biological membranes or “what the cell sees” in the in-vivo context. Hundreds of proteins have been identified to confer a distinct biological identity of nanoparticles in different microenvironments depending on their size, chemistry, and surface modification (recently reviewed in ref. 2). These factors were found to be critical determinants of the biodistribution and pharmacodynamics of nanoparticles. On the other hand, the ability of the surfaces of nanoparticles to partially denature certain corona proteins exposing “cryptic epitopes” highlights the role of the protein corona in the toxicology of nanoparticles4–6. The formation of a protein corona is particularly important in the context of nanoparticle interaction with amyloidogenic peptides such as amyloid-β (Aβ42) and islet amyloid polypeptide (IAPP), which are associated with Alzheimer’s disease (AD) and diabetes mellitus type 2 disease, respectively. Nanoparticles have been shown to catalyze amyloid formation via binding of amyloidogenic peptides in their corona, thereby increasing local peptide concentration and inducing conformational changes that facilitate fibril growth via a heterogenous nucleation mechanism7,8. This surface-assisted (heterogenous) nucleation has been demonstrated for several nanoparticles with different amyloidogenic peptides including IAPP and Aβ42,9,10.

Here we studied viruses in terms of their biophysical equivalence to synthetic nanoparticles in extracellular environments. As nanosized obligate intracellular parasites, viruses lack any metabolic activity outside the cell and can thus be expected to interact with host factors in the microenvironment similar to artificial nanoparticles. In the current work, we used well-established techniques of nanotechnology to study the protein corona of respiratory syncytial virus (RSV) in comparison with herpes simplex virus type 1 (HSV-1) and synthetic liposomes. In addition, we studied the interaction of both RSV and HSV-1 with amyloidogenic peptides.

RSV is an enveloped Orthopneumovirus with a diameter between 100 and 300 nm, and a single-stranded negative-sense RNA genome with 10 genes encoding 11 proteins11. It is a leading cause of acute lower respiratory tract infections in young children worldwide, causing up to an annual estimate of 34 million cases12. By the second year of life, nearly 90% of children get infected with RSV causing up to 196,000 yearly fatalities13. Reinfection with RSV occurs throughout life, usually with mild local symptoms in the upper airways14. However, reinfection in the elderly and immunocompromised individuals can lead to severe clinical disease in the lower airways. Although natural infection leads to the production of neutralizing antibodies, the ability of these antibodies to protect from subsequent RSV infections appears to be incomplete15,16. Neither a vaccine nor an antiviral therapy is yet available, except for passive immunization using the anti-RSV monoclonal antibody palivizumab. Early vaccine trials using formalin-inactivated RSV led to enhanced disease with up to 80% of vaccinees being hospitalized and two dying following natural RSV infection14,16. This led to the hypothesis that host immune responses play an important role in the pathophysiology of airway disease caused by RSV.

HSV-1 is an example of another virus with high prevalence, infecting nearly 70% of the human population17. HSV-1 is a double-stranded DNA virus consisting of an icosahedral nucleocapsid surrounded by tegument and envelope with virion sizes ranging from 155 to 240 nm18. HSV-1 is a neurotropic virus that infects peripheral sensory neurons and establishes latency19. Latent HSV-1 is occasionally reactivated causing peripheral pathology and under certain circumstances it can migrate into the central nervous system causing herpes simplex encephalitis, the most common cause of sporadic fatal viral encephalitis19. In the context of the current work, we focused on the presumptive role of HSV-1 in the pathology of AD. A number of risk factors have been associated with AD, including the E4 allele of the apolipoprotein E (Apo-E), diabetes, vascular pathology, neuroinflammation, and infections20. Several recent studies have supported the theory of a significant role of HSV-1 in the disease21. HSV-1 DNA was found to be localized within amyloid plaques in AD patients and HSV-1 infection has been shown to promote neurotoxic Aβ accumulation in human neural cells and to the formation of Aβ deposits in the brains of infected mice22,23. Moreover, the presence of anti-HSV IgM antibodies, which indicate HSV reactivation, is correlated with a high risk of AD and antiherpetic treatment is correlated with a reduced risk of developing dementia24,25. Despite these correlations, the mechanism by which viruses induce amyloid aggregation, the major pathological hallmark of AD, is not known.

In the present study, we demonstrated that upon encountering different biological fluids, RSV accumulated extensive and distinct protein coronae compared with HSV-1 and synthetic liposomes. The various coronae were dependent on the biological fluid and exerted markedly different effects on RSV infectivity and capacity to activate monocyte-derived dendritic cells (moDCs). Moreover, upon interaction with an amyloidogenic peptide derived from IAPP, RSV accelerated the process of amyloid aggregation via surface-assisted heterogenous nucleation. This amyloid catalysis was also demonstrated for HSV-1 and the Aβ42 peptide in vitro and in an AD animal model. Our findings highlight the importance of viral protein corona interactions for viral pathogenesis and provide a direct mechanistic link between viral and amyloidopathies.

Results
Rich and unique protein coronae for RSV and HSV-1. Based on the extensive literature describing the significant role of corona factors in synthetic nanoparticle functionality, we used established techniques to answer questions regarding RSV pathogenicity26. Using proteomics, we assessed the RSV protein corona profiles in adult human plasma (HP), juvenile (6-month-old infants tested RSV negative at the time of sample collection) HP (JHP), human bronchoalveolar lavage fluid (BALF) from healthy adults, rhesus macaque plasma (MP), and fetal bovine serum (FBS). These biological fluids represent different microenvironments encountered by the virus in terms of tissue tropism (HP vs. BALF), zoonosis (MP), and culturing conditions (FBS). The biological fluids were screened for antibodies against RSV using enzyme-linked immunosorbent assay (ELISA) and both adult HP and BALF contained high levels of anti-RSV IgG antibodies, unlike JHP, MP, and FBS (Supplementary Fig. 1).

Viral stocks were produced in serum-free conditions to prevent initial contamination with FBS proteins. Virions produced under serum-free conditions were incubated with 10% v/v solutions of different biological fluids. Controls included non-infected cell medium representing the background cellular secretome and synthetic lipid vesicles of a size comparable to RSV (200 nm) with positively or negatively charged surfaces. In addition, we compared the coronae of RSV to HSV-1 to probe for differences between different viruses of relatively similar size. After incubation for 1 h at 37 °C, the virions were re-harvested by centrifugation and washed twice before performing mass spectrometry (MS)-based proteomic analyses.
Assessment of the proteomic data by principle component analysis (PCA) showed that RSV and HSV-1 samples were well separated from one another, and that the viral samples were well separated from the control samples with the replicates clustering together (Fig. 1 and Supplementary Fig. 2). Replicate clustering was confirmed via corresponding correlation plots for the samples in the PCA, which showed higher correlation coefficients between the replicates within the same corona condition than between different conditions (Supplementary Fig. 3). Notably, both RSV and HSV-1 possessed distinctive proteomic profiles depending on the biological fluid (Fig. 1c and Supplementary Fig. 2c). In addition, RSV protein corona profiles were different between HP and jHP (Supplementary Fig. 2d); however, the use of a different MS for this experiment prevents direct comparison with the data shown in Fig. 1. The reproducibility of the corona preparations was further assessed by calculating coefficient of variation (CV) between the replicates for each sample type. The CVs ranged between 16% and 42% depending on sample type (average 28%, Supplementary Fig. 3d).

In order to determine whether the most abundant corona factors were the most abundant proteins in the biological fluids, the crude fluids were also analyzed by MS. The top ten most abundant proteins identified are presented in Table 1. Similar to previous findings for nanoparticles, our data showed that the most abundant proteins in the protein corona were not necessarily the most abundant in the biological fluids. This, together with the PCA analyses comparing RSV with HSV-1 and the controls indicated enrichment of particular corona factors depending on the surface properties of the virus. Moreover, proteomic data sets were visualized by heatmap and hierarchical clustering revealing an extensive protein corona signature for viral samples in each biological fluid (Fig. 1d). Although the serum-free viral particles contained some host factors that were incorporated during virus replication and budding from the cells, the virus was able to subsequently accumulate a different set of host factors that were dependent on the biological fluid. As such, a characteristic viral biological identity was associated with each biological fluid. The viral corona factors present on all three replicates in each biological fluid are listed in Supplementary Data 1. In addition, the raw proteomics data are available in Supplementary Data 2.

In addition, we used transmission electron microscopy (TEM) to visualize the viral protein corona. A layer of factors was observed interacting with the viral surface upon encounter with cell membranes, which was absent in serum-free conditions (Fig. 2a). This demonstrated that RSV accumulated a layer of corona factors that are likely involved in cellular interactions. We also performed cryoimmuno-electron microscopy (iEM) using antibodies for certain proteins that were detected in the viral corona proteomic analysis. In serum-free conditions, we used an anti-RSV F-protein antibody. For adult HP and BALF coronae, we used anti-human IgG and anti-surfactant protein A (SP-A) antibodies, respectively. The bound antibodies were detected using secondary antibodies coated with 10 nm gold nanoparticles. As shown in Fig. 2b, corona factors were bound to the surface of RSV and labeled with the respective antibodies. To further

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**Fig. 1** RSV accumulates a rich and distinctive protein corona in different biological fluids. **a-c** Principal component analyses (PCA) of the corona proteomic profiles of RSV, HSV-1, and controls. Triplicate samples were incubated with 10% v/v solutions of each different biological fluid for 1 h at 37 °C, then re-harvested, washed, and finally analyzed by MS. Only proteins significantly detected (FDR 1%) in all three replicates in each condition were used. NI = non-infected supernatant, (−)Lipo = negatively charged lipid vesicles, 200 nm, (+)Lipo = positively charged lipid vesicles, 200 nm. **a** PCA comparing proteomic profiles in human plasma (HP). **b** PCA comparing proteomic profiles in fetal bovine serum (FBS). **c** PCA comparing the corona profiles of RSV in different biological fluids; HP, FBS, MP, or BALF. **d** Heatmap representing the viral corona fingerprints of RSV after incubation in different biological fluids. The three columns in the heatmap show three replicates. Only proteins significantly detected (FDR 1%) in all three replicates in each condition were used. Red and blue indicate higher and lower than the mean protein signal, respectively. Scale bars represent row Z-scores.
Table 1 Top ten proteins in the protein coronae of RSV, HSV-1, and crude biological fluids

| Top ten proteins | HSV-1 + HP | RSV + MP | RSV + FBS | HSV-1 + BALF | RSV + HP | RSV + BALF | HP |
|------------------|-----------|----------|-----------|-------------|----------|-------------|----|
| Beta-actin-like protein 2 [ACTBL_HUMAN] | Ig gamma-1 chain C region [A00A87X079_HUMAN] | Actin, cytoplasmic 1 [ACTB_BOVIN] | Serum albumin [ALBU_HUMAN] | Tyrosine-protein kinase receptor [FIMCMS_BOVIN] | Chromatin assembly factor 1 subunit B [ASD9H4_BOVIN] | Lysozyme C [LYSC_HUMAN] | |
| Actin, cytoplasmic 1 [ACTB_BOVIN] | Complement C3 [C03_HUMAN] | Phosphoprotein [PHOSP_HRSSVA] | Serum albumin [ALBU_HUMAN] | Chromatin assembly factor 1 subunit B [ASD9H4_BOVIN] | Serum albumin [ALBU_HUMAN] | Ig alpha-1 chain C region [IGHA1_HUMAN] | |
| Actin, cytoplasmic 1 [ACTB_BOVIN] | Complement component 3 [A00A06QNP7_BOVIN] | Complement C4-B [CO4B_HUMAN] | Alpha-2-HS-glycoprotein [FETUA_BOVIN] | Lactotransferrin [E7EQB2_HUMAN] | Hemoglobin subunit beta [HBB_HUMAN] | Ig alpha-2 chain C region [IGHA2_HUMAN] | |
| Actin, cytoplasmic 1 [ACTB_BOVIN] | Complement component 3 [A00A06QNP7_BOVIN] | Tubulin alpha-1C chain [TBA1C_HUMAN] | Alpha-2-HS-glycoprotein [FETUA_BOVIN] | Ig lambda-1 chain C regions (Fragment) [A0A075S6L6_HUMAN] | Alpha-1B-glycoprotein [A1BG_BOVIN] | Protein IGKV3-11 [A0A087WZ8_HUMAN] | |
| Actin, cytoplasmic 1 [ACTB_BOVIN] | Complement component 3 [A00A06QNP7_BOVIN] | Nucleoprotein [NCAP_HRSVA] | Ig lambda-1 chain C regions (Fragment) [A0A075S6L6_HUMAN] | Ig gamma-1 chain C region [IGHG1_HUMAN] | Ig lambda-1 chain C regions (Fragment) [A0A087WZ8_HUMAN] | Haptoglobin OS [HPT_HUMAN] | |
| Actin, cytoplasmic 1 [ACTB_BOVIN] | Complement C3-A [C03A_HUMAN] | Tubulin alpha-1C chain [TBA1C_HUMAN] | Uteroglobin [UTER_HUMAN] | Ig gamma-1 chain C region [IGHG1_HUMAN] | Ig lambda-1 chain C regions (Fragment) [A0A075S6L6_HUMAN] | Ig lambda-1 chain C regions (Fragment) [A0A087WZ8_HUMAN] | |
| Actin, cytoplasmic 1 [ACTB_BOVIN] | Complement C3-A [C03A_HUMAN] | Complement C4-B [CO4B_HUMAN] | Actin, cytoplasmic 1 [ACTB_BOVIN] | Ig gamma-1 chain C region [IGHG1_HUMAN] | Ig beta-1 chain C regions (Fragment) [A0A087WZ8_HUMAN] | Ig lambda-1 chain C regions (Fragment) [A0A075S6L6_HUMAN] | |
| Actin, cytoplasmic 1 [ACTB_BOVIN] | Complement C3-A [C03A_HUMAN] | Tubulin alpha-1C chain [TBA1C_HUMAN] | Actin, cytoplasmic 1 [ACTB_BOVIN] | Ig lambda-1 chain C regions (Fragment) [A0A075S6L6_HUMAN] | Ig lambda-2 chain C regions (Fragment) [A0A087WZ8_HUMAN] | Ig lambda-2 chain C regions (Fragment) [A0A075S6K8_HUMAN] | |
| Actin, cytoplasmic 1 [ACTB_BOVIN] | Complement C3-A [C03A_HUMAN] | Tubulin alpha-1C chain [TBA1C_HUMAN] | Actin, cytoplasmic 1 [ACTB_BOVIN] | Ig lambda-1 chain C regions (Fragment) [A0A075S6L6_HUMAN] | Ig lambda-2 chain C regions (Fragment) [A0A087WZ8_HUMAN] | Ig lambda-2 chain C regions (Fragment) [A0A075S6K8_HUMAN] | |
| Actin, cytoplasmic 1 [ACTB_BOVIN] | Complement C3-A [C03A_HUMAN] | Tubulin alpha-1C chain [TBA1C_HUMAN] | Actin, cytoplasmic 1 [ACTB_BOVIN] | Ig lambda-1 chain C regions (Fragment) [A0A075S6L6_HUMAN] | Ig lambda-2 chain C regions (Fragment) [A0A087WZ8_HUMAN] | Ig lambda-2 chain C regions (Fragment) [A0A075S6K8_HUMAN] | |
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confirm the binding of these factors, we performed western blotting analysis on viral particles incubated with either HP or BALF. Several bands were detected for SP-A in the BALF corona samples and were completely lacking in the HP corona samples (Supplementary Fig. 4a). In addition to the main SP-A bands, several high-molecular-weight bands were also detected with the anti-SP-A antibody. This suggested that SP-A was forming complexes either on its own or with other corona factors. When stained with anti-human IgG antibodies, bands were detected in both coronae, but with higher intensity in the HP corona compared with the BALF corona (Supplementary Fig. 4b). The bands were normalized to the RSV G protein band, which was used as a loading control (Supplementary Fig. 4c). Taken together, our data demonstrated that RSV acquired a differential protein corona layer depending on the biological fluid.

Protein corona influences viral infectivity and moDC activation. To investigate whether differential corona composition affects viral infectivity, virions produced under serum-free conditions were pre-incubated with different biological fluids before infection of HEp-2 cells. The RSV virions were incubated with biological fluids at a protein concentration of 0.3 mg/mL (equivalent to 5% v/v) for 1 h at 37 °C then diluted ten times in serum-free medium before infecting the cells at final multiplicity of infection (MOI) of 1. Corona pre-coating had a significant effect on viral infectivity as demonstrated by the differential frequency of green fluorescent protein (GFP)-expressing cells quantified by flow cytometry (Fig. 3a, b and Supplementary Fig. 5). HP corona pre-coating significantly reduced infectivity compared with serum-free conditions, whereas FBS and MP led to five- to sixfold enhancement in infectivity. The BALF corona also enhanced infectivity, but to a lower extent as compared with MP and FBS. Moreover, fluorescence microscopy revealed syncytia formation of cells infected with BALF, MP, and FBS coronae (Supplementary Fig. 6). No significant differences in cell toxicity were observed. jHP pre-coating, on the other hand, slightly enhanced infectivity, but it did not reach significance (Fig. 3b). This lack of inhibition compared with adult HP could be attributed to the lack of anti-RSV antibodies in the selected jHP (Supplementary Fig. 1). Furthermore, we investigated the effect of different coronae on the activation of human moDCs by quantifying the expression of the maturation marker CD86. Differentiated moDCs were infected by virions produced under serum-free conditions with different corona pre-coatings for 4 h in serum-free conditions. The cells were then washed and incubated in serum-containing medium for 72 h before flow cytometry analyses. Only the BALF pre-coated virions were able to induce moDC activation and increase CD86 expression (Fig. 3c–f). Notably, adding BALF alone or corona-free RSV did not activate the cells,
showing that it was the RSV-BALF corona complex that induced moDC activation. In addition, BALF pre-coating enhanced infectivity in moDCs as quantified by flow cytometry of GFP expression (Fig. 3e, f). Altogether, this shows that pre-incubation of virions produced under serum-free conditions in different biological fluids to allow a corona formation greatly affects infectivity and the ability to induce moDC activation in a biological fluid-dependent manner.

**Differential RSV corona composition.** The set of factors that were only present in the HP corona and not in the other
conditions comprised several antibodies and complement factors (Fig. 4a). Moreover, gene enrichment analyses of HP corona revealed an enrichment of immunological Gene Ontology (GO) terms such as complement activation and humoral immune response (Fig. 4b). The HP corona proteomic profile was consistent with the observed inhibition of infectivity (neutralization), indicating that the corona characterization methodology is representative of the actual layer of host factors that surrounds the viral particle. Notably, despite having comparably high levels of virus-specific IgG antibodies (according to ELISA analysis, Supplementary Fig. 1), BALF imparted opposite effects on infectivity compared with HP (Fig. 3). At the proteomics level, GO analysis revealed that the viral BALF corona comprised a different set of factors that are less enriched in immunological components and more enriched in factors related to adhesion, anchoring, protein targeting and localization to membranes, and protein complex binding (Fig. 4). This was further supported by the western blotting analysis where the corona of the BALF-incubated virions was less enriched in human IgG compared with the viral HP corona (Supplementary Fig. 4).

On the other hand, anti-RSV IgG-negative fluids (FBS and MP) enhanced viral infectivity in HEp-2 cells. The effect of both FBS and MP corona on enhancement of RSV infectivity was concentration-dependent as shown in Fig. 5a. Although the FBS corona enhancement increased with concentration, the MP effect decreased after a protein concentration of 0.3 mg/mL was reached. This can be due to the increase of unbound corona factors that compete with bound receptors for cellular receptors. Furthermore, we investigated the effect of corona pre-coating with FBS and MP on viral neutralization via palivizumab, which is a humanized monoclonal antibody directed against the F protein of RSV. We found that the enhancing effects of the coronae were completely lost in the presence of the antibody, and that the antibody neutralization curves were very similar in the presence and the absence of the viral corona (Fig. 5b). These data demonstrated that high-affinity antibodies were able to compete out corona factors to impart host protection. GO analysis revealed that FBS and MP corona were also enriched in terms such as anchoring, adhesion, receptor binding, protein complex binding, membrane organization interspecies interaction, viral process, and mutualism through parasitism (Fig. 5c, d).

**RSV catalyzes amyloid aggregation.** As nanoparticles are known to bind amyloidogenic peptides in their corona leading to induction of amyloid aggregation via a heterogenous nucleation mechanism, we next investigated whether viruses are also capable of this particular corona interaction. We investigated the interaction of RSV with a model amyloidogenic peptide (NNFAGAIL) derived from the IAPP. We traced the ability of RSV to accelerate amyloid kinetics using the well-established thioflavin-T (ThT)-based methodology. The ThT dye changes its fluorescence emission spectrum upon binding to amyloid fibrils and plotting relative changes in the fluorescence intensity against time illustrates the kinetics of the amyloid formation process. Using the ThT assay, we found that the presence of RSV particles significantly accelerated amyloid formation of NNFAGAIL compared with non-infected cell supernatant demonstrating that RSV acted as a catalytic surface for amyloid aggregation (Fig. 6a). As a control, we compared the kinetic curves of the virus-containing medium vs. virus-free medium upon incubation with ThT alone without a peptide. As shown in Supplementary Fig. 7a, the curves were very similar, indicating that the relative changes that we observed in the presence of amyloidogenic peptides are not due to unspecific interaction of the virions with the ThT dye. In addition, extensive fiber networks were observed with TEM within 100 min of incubation with RSV (Fig. 6b) and some virions were located at the tip of these fibers (Fig. 6c). Moreover, RSV catalytic activity was more efficient than lipid vesicles of similar size; however, it was dramatically reduced in the presence of 5% FBS, indicating a competition between the peptide and other corona factors for the viral surface (Fig. 6d). On the other hand, RSV failed to catalyze the amyloid aggregation of GNNQQNY peptide, which is derived from yeast prion protein (Fig. 6e). This, in turn, indicated that such interactions are not universal to all amyloidogenic peptides but display some selectivity depending on the virus/peptide pair.

**HSV-1 catalyzes Aβ_{42} amyloid aggregation in vitro and in vivo.** The finding that viral surfaces could serve as catalysts for amyloid formation warranted further investigation and confirmation using another virus/peptide system. To this end, we investigated HSV-1 and the Aβ_{42} peptide, whose aggregation is a major hallmark of AD. Recently, there has been an increasing body of reports suggestive of a correlation between HSV-1 infection and AD, reviewed in ref. 21. However, evidence of a direct role of HSV-1 in the process of amyloid nucleation and subsequent fibril growth is currently lacking.

We found that HSV-1 significantly accelerated amyloid formation of Aβ_{42} compared with non-infected cell supernatant (Fig. 7a). As a control, and similar to the RSV/NNFAGAIL system, both the virus-containing medium and virus-free medium produced similar curves upon incubation with ThT alone without peptide (Supplementary Fig. 7b). The catalytic activity was reduced by the presence of FBS, also indicating a competition at the viral surface (Fig. 7b). In addition, HSV-1 was more efficient than liposomes in accelerating the amyloid aggregation kinetics (Fig. 7c). Furthermore, the propensity of HSV-1-mediated amyloid catalysis was higher for the more
amyloidogenic Aβ42 peptide compared with the shorter, less amyloidogenic Aβ40 peptide (Fig. 7d). Amyloid induction was further confirmed by TEM demonstrating fibril formation within 100 min of incubation with viral particles (Fig. 7e–h). Amyloid protofilaments and fibrils at different stages of elongation were observed interacting with the viral surface. Figure 7e and f show brillary structures emerging from one viral particle, supported by TEM demonstrating a nucleation mechanism was taking place on the multiple brillary structures that are part of an extensive network of fibrils at different stages of elongation were observed interacting with the viral surface. Figure 7e and f show brillary structures emerging from one viral particle, supported by TEM demonstrating that a nucleation mechanism was taking place on the surface, thereby sparking fibril elongation. Viral particles also interacted with fibrillar structures that are part of an extensive network of fibers as shown in Fig. 7g, h. Importantly, to demonstrate the in-vivo relevance of our mechanistic findings, we intracranially infected transgenic 5XFAD mice with HSV-1. The 5XFAD mouse is a widely used AD model, as it recapitulates many AD phenotypes with rapid onset of Aβ aggregation that spreads to the hippocampus and cortex by 6 months of age. We observed a significant increase in Aβ42 accumulation in the hippocampi and cortices of HSV-1-infected mice 48 h post infection (Fig. 7i) in comparison with animals injected with non-infected supernatant. Representative images of the amyloid staining demonstrate the dramatic difference in amyloid accumulation between infected and non-infected animals (Fig. 7j). These results validated our biophysical findings using the ThT assay and demonstrated that the viral corona-driven catalysis of amyloid aggregation could take place in in-vivo situations.

Discussion

Viruses rely on the cellular machinery of the host for replication, production of viral proteins, assembly, and export out of the cell. However, outside cells, viruses share many biophysical properties with artificial nanoparticles. Based on this biophysical equivalence, we hypothesized that viruses accumulate a rich and selective protein corona layer in the extracellular environment similar to nanoparticles. Examples of particular host factors that bind to viral surfaces have previously been described. For example,
lipoproteins such as Apo-E were shown to be essential for hepatitis C virus infection. Interestingly, amyloids derived from prostatic acidic protein were shown to contribute to the infectivity of lentiviral vectors when pseudotyped with multiple types of envelope proteins. Furthermore, soluble heparin sulfated proteoglycans were shown to enhance the infectivity of human papillomavirus. Interestingly, amyloids derived from prostatic acidic phosphatase in semen were shown to enhance HIV-1 infectivity by several orders of magnitude. In addition, plant viral nanoparticles that are used for drug delivery were shown to possess a protein corona that affects their uptake and biodistribution. However, to our knowledge, there is no previous work that has characterized the protein corona of an infectious animal virus in different biological fluids and studied its effect on viral pathogenicity.

In this work, we used proteomics to study viral protein corona enrichment in different biological fluids. We investigated the RSV protein corona in biological fluids that are relevant to viral tropism, zoonosis, and culturing conditions. As RSV is a virus tropic to the respiratory tract, we compared the viral corona in fluids that are relevant to viral tropism, zoonosis, and culturing conditions. As RSV is a virus tropic to the respiratory tract, we compared the viral corona in fluids that are relevant to viral tropism, zoonosis, and culturing conditions. As RSV is a virus tropic to the respiratory tract, we compared the viral corona in fluids that are relevant to viral tropism, zoonosis, and culturing conditions. As RSV is a virus tropic to the respiratory tract, we compared the viral corona in fluids that are relevant to viral tropism, zoonosis, and culturing conditions. As RSV is a virus tropic to the respiratory tract, we compared the viral corona in fluids that are relevant to viral tropism, zoonosis, and culturing conditions.
studied the corona in FBS, which is the most commonly used cell growth supplement for viral production and in-vitro studies. We compared the RSV corona with HSV-1 and lipid vesicles of similar size with positively or negatively charged surfaces, as well as the biological fluids per se. PCA, quantitative intra-sample variability analysis, and correlation matrices demonstrated that the RSV corona profiles were well separated from that of HSV-1 and lipid vesicles (Fig. 1 and Supplementary Figs. 2 and 3). Intra-sample variability could arise in the process of sample preparation during incubation, washing, and digestion or during mass spectroscopy-based detection.

Notably, although the viral corona profile was dependent on the biological fluid, it was not a mere reflection of the most abundant proteins, as demonstrated earlier for nanoparticles and as shown in the Table 1 of the top ten proteins present in the viral coronae in comparison with crude biological fluids. The viral protein corona was visualized using TEM, which revealed a layer surrounding the viral surface involved in cellular interactions (Fig. 2a). Using iEM and western blotting, we could detect specific proteins identified from the proteomic analysis (human IgG in HP and SP-A in BALF) associated with RSV upon incubation with HP or BALF, respectively (Fig. 2b and Supplementary Fig. 4). Functionally, the coronae from the different biological fluids enhanced RSV infectivity, except for the HP corona, which completely neutralized the virus (Fig. 3). Taken together, using several complementary methods, our data demonstrated the specific enrichment of differential corona profiles on viral surfaces. As such, the protein corona represents an initial phase of viral–host interactions that precedes cellular interaction and affects subsequent infectivity. Unlike the viral genome-coded surface proteins, the viral protein corona is an acquired structural layer that is dependent on the viral microenvironment resulting in different viral identities based on the target tissue and the target organism. Moreover, as the corona layer is a rich and complex layer, the final biological effect is expected to be dependent on a multitude of corona factors rather than a single protein.

Proteomic analysis of the HP corona revealed that it was enriched in antibodies and complement factors, which could explain the neutralization effect (Figs. 3 and 4). Other factors of documented immunological functions such as fibrinogen, properdin, annexin A1, protein S100, and vimentin were also detected in the HP corona (Supplementary Data 1). These factors could be parts of an immunological corona that enables efficient viral neutralization and/or immune system modulation. On the other hand, jHP, which lacked the anti-RSV antibodies, failed to neutralize the virus and the jHP corona was less enriched.
in immunological factors compared with the HP corona (Table 1 and Fig. 3). Interestingly, the BALF corona enhanced viral infectivity in both HEp-2 cells and moDCs, despite having equally high anti-RSV IgG antibody titers as compared with HP according to ELISA analysis (Supplementary Fig. 1). The proteomic and GO analysis showed that the BALF corona was less enriched in immunological factors (Fig. 4). In addition, less IgG was detected in the BALF corona in the western blotting analysis (Supplementary Fig. 4). The failure of BALF to neutralize the virus despite being rich in anti-RSV antibodies suggested that the differential protein corona profile could affect antibody binding in BALF conditions. This might explain the recurrence of pulmonary RSV infection even in individuals with high IgG titers. An alternative explanation is that the BALF anti-RSV antibodies are of lower affinity than their HP counterparts and are thus less able to compete out other corona factors that enhance viral infectivity.
infectivity. It remains to be elucidated which avidity is required in order to compete out the corona layer. The list of proteins that were uniquely detected in the BALF corona included pulmonary surfactants that are known to enhance RSV infectivity, nucleolin, and epidermal growth factor receptor (EGFR), which were shown to be important for RSV cell entry. Adhesion molecules (such as tetraspanin, neuroplastin, integrin, and cadherin), coxsackievirus/adenovirus receptor, poliovirus receptor, and zinc finger CCCH-type antiviral protein 1 (Fig. 4). Notably, pre-incubation of RSV with BALF was the only condition that induced moDC activation (Fig. 3). Importantly, it was not the combination of the proteins in the biological fluids or the virus per se that affected the outcome, but rather the virus–corona complex, as shown by the lack of moDC activation by BALF or RSV alone. Moreover, and in accordance with the documented role of corona factors in nanoparticle immunogenicity, our results demonstrated the role of the acquired corona layer in virus-induced immune activation that might contribute to disease pathophysiology. It can be speculated that the viral corona factors are part of the pathogen-associated molecular patterns, which are recognized by the innate immune system. It also suggests that corona factors need to be considered in the design of vaccines and adjuvants for efficient stimulation of the immune system.

Additional biological fluids investigated in our study included FBS and MP, and they both enhanced the infectivity of RSV in a concentration-dependent manner (Fig. 5a). Artificially adding anti-RSV antibodies (palivizumab) successfully neutralized the virus even in these corona conditions, indicating successful outcompetition by the antibody (Fig. 5b). GO analysis suggested a role of FBS and MP corona factors in interspecies interaction, mutualism, viral process, protein complex binding, unfolded protein binding, anchoring, and adhesion (Fig. 5c, d). Factors uniquely present in the FBS corona that may contribute to these effects include C4b-binding protein α-like, which is a complement inhibitor, isoform 2 of fermitin family homolog 2, which binds to membranes enriched in phosphoinositides and enhances integrin-mediated adhesion and hepatitis A virus cellular receptor 1 (Fig. 5c). In the MP corona, several adhesion proteins were found including the following: fibronectin isoform 4 pre-proprotein, endothelial cell-selective adhesion molecule, fermitin family homolog 3 short form, and zyxin. The MP corona also contained receptor ligands such as the following: transferrin and C-X-C motif chemokine together with tetherin (isoform 2 of bone marrow stromal antigen 2), which possesses antiviral properties. Taken together, this illustrated that the observed functional effects of the viral protein corona were most likely mediated by a combination of factors that are enriched on the viral surface and not by a single factor. As many viruses bind to several receptors and co-receptors, such interactions might be taking place in a multivalent manner. In addition, our results also highlight that the viral protein corona has to be taken into consideration in relation to zoonosis and applications involving viral propagation in vitro.

We then investigated whether viral corona interactions with host factors involve surface-assisted nucleation of amyloids. Nanoparticles have been shown to act as catalytic surfaces that facilitate heterogenous nucleation of amyloid fibrils via binding, concentrating, and enabling conformational changes of amyloidogenic peptides. Similar to what has been reported with nanoparticles, we found that RSV accelerated the kinetics of amyloid aggregation of a model amyloidogenic peptide derived from IAPP (NNFGAIL). This demonstrated that viral particles are also capable of amyloid catalysis via surface-assisted heterogenous nucleation (Fig. 6). In order to investigate whether this catalytic mechanism extends to other virus-amyloid pairs, we evaluated whether HSV-1 could accelerate the amyloid kinetics of Aβ42, which is implicated in AD. Several recent studies have suggested HSV-1 involvement in AD. Herein, we found that HSV-1 accelerated the kinetics of amyloid aggregation of Aβ42 and, to a lesser extent, the aggregation of Aβ40 (Fig. 7). HSV-1 was more efficient than lipid vesicles in amyloid catalysis and this efficiency decreased in the presence of FBS demonstrating a competition with other corona factors on the viral surface. In addition, TEM demonstrated an interaction between amyloid fibrils at different stages of maturation and the viral surface via early protofibrillar intermediates, which we speculate represent the surface-assisted nucleation process. Importantly, infecting 5XFAD AD animal models with HSV-1 lead to increased accumulation of Aβ42 plaques as evident by immunohistochemical analysis (Fig. 7), demonstrating that the viral corona-driven catalysis can take place in vivo.

Taken together, our data on RSV and HSV-1 demonstrated that viruses can physically act as nano-surfaces capable of catalyzing amyloid nucleation and leading to accelerated fibril formation. Although our results do not prove disease causality, they present mechanistic explanation of the clinical and experimental correlations drawn between HSV-1 and AD, which requires further investigation. Interestingly, Apo-E, which is a well-known risk factor for AD, was enriched in the HSV-1 corona, suggesting even further disease links (Supplementary Data 1).

Several recent studies have suggested that Aβ42 is an antimicrobial peptide that aggregates to sequester pathogens. Here we present an alternative but not mutually exclusive explanation. Our corona-driven hypothesis suggests that the virus interacts with extracellular amyloidogenic peptides as part of the pathogenesis and the bound peptides do not necessarily possess an immunological and/or an antimicrobial function. This is further corroborated by studies showing that amyloids can enhance...
the infectivity for viruses such as HIV and HSV^{35,58}, and the fact that amyloid precursor protein knockout animals are not more susceptible for infections compared with wild-type animals^{59}. From this perspective, virus surface-assisted nucleation might have evolved as a mechanism by which viruses modulate the host’s extracellular environment by catalyzing the phase transition of certain peptides from soluble to insoluble forms; a phase change that could lead to toxic gain or loss of function, or both. In addition, the viral surface-assisted nucleation mechanism could be extended to other viruses correlated with neurodegenerative pathologies such as HIV and HIV-associated neurocognitive disorder^{60}, and influenza virus and post-encephalitic parkinsonism^{61}, and others. Furthermore, the phenomenon of amyloid polymorphism^{62} might in part be related to the different nucleation mechanisms, where we hypothesize that the virus-catalyzed heterogeneously nucleated amyloids would be morphologically distinct from homogeneously nucleated amyloids that result from aberrant protein expression/overexpression. This hypothesis can be tested in future studies on the Aβ polymorphism in familial and sporadic AD^{63}, and whether this structural polymorphism can be traced back to different nucleation mechanisms related to different etiologies. Finally, the implication that viruses are capable of inducing conformational changes in bound host factors leading to exposure of cryptic epitopes may prove important for better understanding the correlation between viruses and autoimmune diseases.

To conclude, the current work was based on the biophysical equivalence between viruses and artificial nanoparticles in extracellular environments. We here demonstrated that nano-technological concepts such as the protein corona and surface-assisted nucleation can be extended to infectious viruses. We showed that viral protein corona accumulation and amyloid catalysis are two aspects of the same phenomenon, namely viral surface interaction with extracellular host proteins. This phenomenon leads to the modulation of how viruses interact with cells and/or the induction of conformational changes in bound proteins that leads to accelerated amyloid aggregation. These findings highlight the potentially critical role of viral extracellular interactions in viral infectivity and in relation to extracellular protein pathology.

Method

Viral and cell culture. HEP-2 cells, a human laryngeal carcinoma cell line, were used for RSV culture and infectivity experiments. Preparation of RSV-A2-GFP stocks was performed using VP-SFM serum-free, ultra-low protein medium containing no proteins, peptides, or other components of animal or human origin (ThermoFisher, USA). HEP-2 cells were initially seeded in growth medium (Dulbecco’s modified Eagle’s medium (DMEM)) with 5% FBS (ThermoFisher, USA), 1% Penicillin/Streptomycin (ThermoFisher, USA), and 0.01 M HEPES (Sigma-Aldrich, Germany) until they reached ~70–80% confluency. At the day of infection, the cells were washed twice with warm phosphate-buffered saline (PBS) and the medium was replaced with VP-SFM containing 0.5% carboxymethyl cellulose (Sigma-Aldrich) was added onto VERO cells for 1 h at 37 °C, then inoculum was removed and fresh medium containing 0.5% carboxymethyl cellulose (Sigma-Aldrich) was added. Cells were fixed and stained 2 days later with a 0.1% crystal violet solution and the number of plaques was counted.

Human BALF samples. The use of human BALF samples for the current study was approved by the Regional Committee for Ethical Review in Stockholm (D. No 2016/1985–32). All donors had given oral and written informed consent to participate in the bronchoscopy procedure with the Helsinki Declaration. Healthy male and female subjects were recruited to participate in the study with the Helsinki Declaration. Healthy male and female subjects were recruited to participate in the study. BALF samples were collected in BALF tubes (Vacutainer, Becton Dickinson, USA) containing 6.6 × 10^9 RSV genome equivalents or serum-free DMEM produced by the Regional Committee for Ethical Review in Stockholm. The obtained BALF was concentrated using Centrifugal Filter Devices (Millipore, Billerica, MA, USA).

Lipid vesicle preparation and characterization. 1,2-Distearoyl-sn-glycerol-3-phosphocholine (DSPC), 1,2-distearoyl-sn-glycerol-3-phospho-(1’-rac-glycerol) (DSPG), and cholesterol were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). The lipids were dissolved in chloroform in molar ratios of 9:1 (DSPC/cholesterol) for neutral liposomes, 9:1 (DSPC/DOPAT) for cationic liposomes, and 9:1 (DSPC/DSPG) for anionic liposomes. The liposomes were formed by the thin film hydration method followed by extrusion through a polycarbonate membrane. Briefly, chloroform was evaporated by heating the sample tube to 65 °C and gradually reducing the pressure to 5 mbar. The liposomes were sonicated in a vacuum rotary evaporation system (Büchi R-114, Büchi Labortechnik AG, Flawil, Switzerland). The resulting thin lipid layer was hydrated with 500 μL of PBS by gently stirring the tube in a water bath (65 °C) for 1 h. The sample was then extruded 11 times (65 °C) through polycarbonate membrane (pore size of 200 nm) with a syringe extrusion device (Avanti Polar Lipids). In which the sample was quickly cooled down and stored in a refrigerator.

Protein corona proteomics. FBS was obtained commercially (ThermoFisher, USA). HP was obtained and pooled from at least three different healthy donors. HP was obtained and pooled from at least three different healthy donors. HEP-2 cells were then washed twice with warm phosphate-buffered saline (PBS) and the medium was replaced with VP-SFM containing 0.5% carboxymethyl cellulose (Sigma-Aldrich). The obtained BALF was concentrated using a Centrifugal Filter Device (Millipore, Billerica, MA, USA).

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incubated with 10% v/v solutions of biological fluids at similar conditions. After incubation, viral/nanoparticle corona complexes were spun at 20,000 x g at 4 °C for 1 h, supernatant removed, and the pellet resuspended in 1 mL PBS. The pellet was washed twice with PBS using the same centrifugation conditions, then boiled at 95 °C for 5 min before measuring the protein content using Micro BCA® protein assay kit (ThermoFisher, USA). The viral/nanoparticle corona complexes were then resuspended in PBS and adjusted with ammonium bicarbonate to a final concentration of 20 mM. The samples were reduced by addition of 1:20 v/v 100 mM dithiothreitol for 45 min at 56 °C and alkylated with 1:20 v/v 100 mM iodoaceta-

Mann–Whitney test was used followed by

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The corona-pre-coated viruses were then added to the cells in serum-free condition (diluted 10×) at a MOI of 1. 24 h post infection, the medium was changed to growth medium, and cells were visualized with fluorescence microscopy (Zeiss Observer A1m, Germany). For TEM of viruses with antibodies, 100 µL of RSV (3 x 10^6 PFU/mL) were incubated with 50 µL 1 mM NNFGAIL (for RSV) or 0.5 µM Vero grams (diluted 10×) at a MOI of 1 h before adding the primary antibodies and negative controls and the kit manufacturer.

**RSV infectivity.** HEp-2 cells were seeded in maintenance medium until they reached 50–60% confluency. On the day of infection, the cells were washed twice with PBS until the supernatant was clear and then replaced with VP-SFM with gentamycin before infecting with RSV at MOI 100 in serum-free conditions or in 5% v/v of different biological fluids. Cells were then fixed and stained with 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.4 at RT for 30 min. TheFixed samples were then rehydrated in ethanol followed by acetone and embedded in LX-112 (Leica, Germany) at 1–2 µm thickness. Digitally imaged using a Tecnai G2 Bio TWIN (FEI Company, Eindhoven, The Netherlands) at 100 kV. Digital images were captured using Frame Grabber (Sony) and Point GRIP software (INOVA, Stockholm, Sweden). Stained sections were then visualized with uranyl acetate followed by lead citrate and imaged in a Tecnai 12 Spirit Bio TWIN TEM (FEI Company, Eindhoven, The Netherlands) at 100 kV. Digital images were captured by a Veleta camera (Olympus Soft Imaging Solutions GmbH, Münster, Germany). For TEM of viruses with antibodies, 100 µL of RSV (3 x 10^6 PFU/mL) were incubated with 50 µL 1 mM NNFGAIL (for RSV) or 0.5 µM Vero in a 100 µL droplet of 2% normal goat serum solution (Sigma, St. Louis, MO) for 1 h before removing the antibody and negative controls and the kit manufacturer.

**Enzyme-linked immunosorbent assay.** The detection of specific anti-RSV IgG antibodies in biological fluids was performed using Human Anti-Respiratory syncytial virus IgG ELISA Kit (ab108765, abcam, UK), according to the manufacturer’s protocol. All biological fluids (PBS, HP, MP, and BALF) were diluted to a protein concentration of 0.3 mg/mL before performing the assay and results were compared with the positive, cutoff and negative controls provided by the kit manufacturer.

**Modulation of endosome acidification.** Human monocytes were negatively selected from buffy coats using the RosetteSep Monocyte Enrichment Kit (1 mL/10 mL buffy coat; StemCell Technologies) and differentiated into moDC, using granulocyte-macrophage colony-stimulating factor (250 ng/mL; PeproTech) and interleukin-4 (IL-4) at a 1:1 ratio. For each technical replicate, 50,000 monocytes were added in 1 mL 2% fetal bovine serum (FBS) diluted to a protein concentration of 0.3 mg/mL before performing the assay and subsequent calculation of FCP-positive cells within the viable cell population. For experiments with palivizumab, cells were treated with different concentrations of the antibody before infection.

**Electron microscopy.** For sections with RSV, HEp-2 cells were seeded in 6 cm dishes in maintenance medium until 70–80% confluent, then washed and medium replaced with VP-SFM with gentamycin before infecting with RSV at MOI 100 in serum-free conditions or in 5% v/v of different biological fluids. Cells were then fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.4 at RT for 30 min. TheFixed samples were then rehydrated in ethanol followed by acetone and embedded in LX-112 (Leica, Germany) at 1–2 µm thickness. Digitally imaged using a Tecnai G2 Bio TWIN TEM (FEI Company, Eindhoven, The Netherlands) at 100 kV. Digital images were captured by a Veleta camera (Olympus Soft Imaging Solutions GmbH, Münster, Germany). For TEM of viruses with antibodies, 100 µL of RSV (3 x 10^6 PFU/mL) were incubated with 50 µL 1 mM NNFGAIL (for RSV) or 0.5 µM Vero in a 100 µL droplet of 2% normal goat serum solution (Sigma, St. Louis, MO) for 1 h before removing the antibody and negative controls and the kit manufacturer.
Western blotting. Serum-free produced RSV was incubated with BALF or HP, both at a protein concentration of 0.1 mg/mL at a ratio of 1:1 v/v (in citrate-buffered saline) and collected and washed similar to the procedure for corona proteomic analysis. Samples were lysed using a RIPA lysis buffer with protease inhibitors and protein content was measured. Samples were heated with loading buffer to 95°C for 5 min and 1 μg of protein was loaded onto NuPAGE® Bis-Tris 4–12% gels then separated at 150 V for 135 min at RT using 1x NuPAGE® MES SDS running buffer (Invitrogen). The gels were then transferred onto nitrocellulose membranes using the iBlot® system (Invitrogen) and, subsequently, the membranes were blocked with Odyssey Blocking Buffer (LI-COR Biosciences GmbH) for 1.5 h. Membranes were incubated overnight at 4°C with anti-SP-A antibody [6F10] (ab51891, Abcam) diluted at 1:500, then probed the next day using goat anti-human IgG (IRDye 800, red, LI-COR Biosciences) and goat anti-mouse IgG (IRDye 680, green, LI-COR Biosciences). To normalize the bands to a loading control, scanned membranes were stripped for 10 min at 65°C using 1x New Blot Nitro Stripbing buffer (LI-COR Biosciences), blocked for 1.5 h, and then incubated with anti-SP-A antibody (ab20747, Abcam) diluted at 1:500 dilution for 2 h at RT before probing with secondary antibody (donkey anti-goat, IRDye 680, green, LI-COR Biosciences). All western blotting signals were scanned using the Odyssey Imager (LI-COR Biosciences GmbH) and quantification was performed on the images using ImageJ software.

Animal experiments. To evaluate the impact of the HSV-1 on brain β-amyloid (Aβ) levels, 3-month-old transgenic 5XFAD mice (purchased from Jackson Laboratories, Bar Harbor, Maine, USA) were randomly divided into two groups. The animals were injected either with the HSV virus or non-infected supernatant using a micro-infusion pump (The Harvard Apparatus Pump Series, Harvard Apparatus, USA) into the right lateral ventricles. Briefly, surgical anesthesia was induced with 5% isoflurane and maintained with 1.8% isoflurane (in 30% O2/70% N2O). The temperature of the animals was maintained at 37 ± 0.5°C using a thermostatically controlled heating blanket connected to a rectal probe (PanLab, Harvard Apparatus, Barcelona, Spain). The skin was opened and the scull exposed. A small hole ~1 mm in diameter was drilled into the following coordinates: ml (medial/lateral) ~ +1.1 mm, a/p (anterior/posterior) ~ −0.3 mm, d/v (dorsal/ventral) ~ −2.0 mm. The mice were infused with either 10 μL of HSV-1 virus (2 × 10^8 PFU/mL) or with non-infected supernatant as control. After injection, the wound was sutured and the animals placed in individual cages to recover for 48 h. All animal work was approved by the Animal Care and Use Committee of the University of Eastern Finland (Kuopio) and performed according to the guidelines of National Institutes of Health for animal care. Mice were killed at 48 h after ICV (intracerebroventricular) infusion for tissue collection. The mice were anesthetized with an overdose of Avertin followed by transcardial perfusion with heparinized saline (0.1 U/mL) and 4% paraformaldehyde to wash similar to the procedure for corona proteomic analysis. Samples were lysed using a RIPA lysis buffer with protease inhibitors and protein content was measured. Samples were heated with loading buffer to 95°C for 5 min and 1 μg of protein was loaded onto NuPAGE® Bis-Tris 4–12% gels then separated at 80 V for 42 min at 4°C using 1x NuPAGE® MES SDS running buffer (Invitrogen). The gels were then transferred onto nitrocellulose membranes using the iBlot® system (Invitrogen) and, subsequently, the membranes were blocked with Odyssey Blocking Buffer (LI-COR Biosciences GmbH) for 1.5 h. Membranes were incubated overnight at 4°C with anti-RSV antibody (ab20747, Abcam) diluted at 1:500 dilution for 2 h at RT before probing with secondary antibody (donkey anti-goat, IRDye 680, green, LI-COR Biosciences). All western blotting signals were scanned using the Odyssey Imager (LI-COR Biosciences GmbH) and quantification was performed on the images using ImageJ software.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.
