Molecular Characterization of PB1-F2-Mediated Pathology in Influenza Mouse Model of Infection

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ABSTRACT

PB1-F2 is a virulence factor of influenza A virus (IAV) known to increase viral pathogenicity in mammalian host. PB1-F2 is an intrinsically disordered protein displaying a propensity to form amyloid-like fibers in IAV-infected cells. Using synchrotron Fourier-transform infrared (FTIR) spectroscopy, we previously evidenced the presence of PB1-F2 fibers in IAV-infected cells and assigned an IR β-aggregated signature at the single-cell level. Using DUV (Deep Ultraviolet) microscopy and taking advantage of the high content of tryptophan residues in the sequence of PB1-F2 (5/90 aa), we showed that the increase of the autofluorescent signal recorded in IAV-infected cells can be correlated with the IR detection of β-aggregates. Here, we used FT-IR and DUV microscopies to prove the presence of PB1-F2 fibers in IAV-infected mice. Mice were infected with a wild-type IAV and its PB1-F2 knockout mutant and monitored at different time post-infection. DUV microscopy was used to map the presence of PB1-F2 β-aggregates within slices of lung tissues of IAV-infected mice. IR spectra were recorded in the regions of interest and subjected to multivariate analysis revealing the presence of β-aggregated structures in mice infected with PB1-F2-expressing IAV. In order to study the correlation between PB1-F2 structure and inflammatory response, NF-KB luciferase transgenic mice were intranasally instilled with monomeric, fibrillated or C- and N-terminal domains of recombinant PB1-F2. Our results clearly show the pro-inflammatory effect of fibrillated PB1-F2 compared to monomeric and non-fibrillated forms. It is noteworthy that only the N-terminal part of PB1-F2, unable to fibrillate, does not provoke any inflammation. Thus, the PB1-F2-induced inflammation is tightly correlated with sequence and oligomerization status of the protein.

Toward Structural Study of HCV Core Protein at SOLEIL

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ABSTRACT

The hepatitis C virus (HCV) controls the lipid metabolic pathways of the host cell to carry out its viral cycle. The virus highjacks cell lipid droplets (LD), cellular structures for lipid storage. LDs serve as source of energy and lipids, but also as an assembly platform for the viral particle [1]. LDs have an original organization with a neutral lipid core, surrounded by a monolayer of phospholipids and various proteins. We focus our research in a particular class of LD proteins, the Class I proteins, which includes the core protein of HCV. These proteins exhibit structural convergence with a hairpin folding. Very few structural data exist on these proteins. They are very difficult to handle due to their high hydrophobicity. Thanks to our skills acquired on the study of oleosins, the class I proteins of LD oilseeds [2-4], we propose a functional and structural study of the core protein of HCV. We developed an original heterologous expression protocol using the yeast model and designed approaches using synchrotron radiation. The results from this project will contribute to a better understanding of the mechanisms developed by the virus to divert host cell lipid metabolism and provide new tools for the development of antiviral strategies.

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