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Mechanism underlying nanoparticle exposure triggered herpesvirus reactivation and translational study into epidemiological cohort

Lianyong Han Research Group Dynamics of Pulmonary Inflammation Institute of Lung Health and Immunity, Comprehensive Pneumology Center (CPC) Helmholtz Zentrum München, Germany

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NP triggered virus reactivation assessed by lytic MHV-68 protein detection

Light-sheet fluorescence microscopy (LSFM):

Overview of MHV-68 lytic viral protein expression in whole lung lobes, during acute infection (day6) and CNP triggered reactivation at latency (day28 + day1)





Acute infection

The localization of herpesvirus reactivation in lung

Main population of macrophages in lungs



Light sheet imaging of whole lung lobes (*black CNP particle disturbs imaging*):

LPS induce herpesvirus reactivation in **CD11b+ infiltrating cells**



> NP exposure triggers lytic MHV-68 protein expression in CD11b+ infiltrating macrophages

Immunofluorescence staining for MHV-68 lytic proteins plus cell specific markers uncovers co-localization with **CD11b+** (infiltration monocytes/macrophages)



Most lytic MHV-68+/CD11b+ cells are also positive for other markers of inflammatory recruited monocytes / macrophages (**GPNMB, ARG1, IBA1**) but not tissue resident macrophages.







> NP exposure triggers lytic MHV-68 protein expression not in CD11c+ alveolar macrophages

Lung tissue



BAL cells



CD11c anti-MHV-68 DAPI

Mechanism of NP triggered gammaherpesvirus reactivation ?

Persistently MHV-68 infected (CD11b+) macrophage cell line (Ana-1/MHV-68) as model for reactivation

*

LPS

DWCNT



Lytic virus protein expression



CNP & DWCNT trigger mitogen associated protein kinase (MAPK) signaling in Ana-1/MHV-68 cells



Time course of MAPK activation

Investigating transcriptomic changes by **Affymetrix Microarray analysis**



No characteristically pro-inflammatory polarization of macrophages by CNP/CNT exposure!

NPs stimulate MAPK pathways in Ana1-BMDMs but no pro-inflammatory signature

- CNPs induced reactivation depends on p38 MAPK signaling



SB203580 inhibits p38 signaling







Plaque assay, 72 h



In vivo experiment setup



Pretreatment with p38-MAK inhibitor SB203580 reduces CNP induced gammaherpesvirus reactivation in the lung of mice



Lytic viral protein staining shows SB203580 reduces CNP triggered herpesvirus reactivation in lung

Human relevance?

Translation of animal experimental data into epidemiological cohorts



Holger Annette Schulz Peters

- **KORA:** Cooperative Health Research in the Augsburg Region, a population-based adult cohort study in the Region of Augsburg, Southern Germany with 2,278 participants in the second follow-up FF4 (2013/14).
- Particulate matter < 2.5 μm (PM 2.5), nitrogen dioxide (NO2), particle number concentration (PNC)
- Epstein-Barr virus (EBV), Human Herpesvirus 6 (HHV6) and Cytomegalovirus (CMV)



Association of high HHV6-titers with high particle number concentration (PNC)



- 1. High Human Herpesvirus 6 (HHV-6) titer is associated with high environmental particle number concentration (PNC).
- 3. In the murine MHV-68 model, CNP induced MHV-68 reactivation, mainly localized to CD11b+ infiltrating macrophage-like cells.
- 3. CNP triggered MHV-68 reactivation via a p38 MAPK dependent signaling, and protective p38 inhibition significantly attenuated

MHV-68 reactivation induced by CNP, and potentially serves as therapeutic target of exacerbations of CLDs.



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Thank you.