Nanoparticles and persistent virus infection – a dangerous liaison for the development of chronic lung disease(s)?

Tobias Stöger
Herpesviruses and lung disease

- Double-stranded DNA-viruses (α, β, γ- herpesviruses)
  γ: EBV (Epstein-Barr V.), Kaposi’s sarcoma-associated herpesvirus

- Complex „life-cycle“: acute/lytic & latent infection

- Latency: life-long virus persistence inside host cells

modified from Bollard et al, 2012
Herpesviruses and lung disease

- Herpesviruses belong to the **most prevalent and persistent known pathogens**, with the majority of healthy people harboring multiple latent herpesviruses (White et al, 2011)

- Herpesvirus infection **alters the host immune response** to non-herpesviral antigens and might trigger collateral damage (White et al, 2011)

- Elevated levels of **Herpesvirus-DNA found** in lung tissue of patients with **pulmonary fibrosis** (Tang et al, 2003)
Virus latency and Nanoparticles as second hit?

Environmental NP and herpesviruses are omnipresent in human society and both, NP-inhalation and persistent herpesvirus-infection are potentially associated with chronic lung disease.

**HYPOTHESIS:**
Exposure to nanoparticles might disrupt the control of viral latency and induce virus reactivation.
Nanoparticles used in this study

Nanoparticles (NP): *carbonaceous nanoparticles*

**Carbon Black**
- Reinforcing filler (in rubber products), color pigment
- In this study: Printex90 (= Ptx90) (Stoeger 2006, 2009; Ganguly 2009, 2011; Götz, 2011)

**Carbon Nanotubes**
- Composite fibers in polymers or building materials
- Cylindrical nanostructures of graphene (=one-atom-thick sheets of carbon)
- In this study: double walled carbon nanotubes (= DWCNT) (Tian 2013; Hirn & Habel in preparation)
Transient inflammation caused IT exposure to spherical NPs (CNP) but not double-walled carbon nanotubes (CNT)

[BAL Inflammatory Cell Counts (10E3)]

Days After Particle or MHV-68 Challenge

50 µg/mouse (IT)
Murine gammaherpesvirus 68 (MHV-68) as a model

= murine gammaherpesvirus 68

• Pathogen of wild murid rodents
• gamma2-herpesvirus, related to primate gammaherpesviruses
• Small animal model for the pathogenesis of gammaherpesviruses
• Induces pulmonary fibrosis in (genetically) susceptible mouse lines
Results *in vitro* (murine cells)
In vitro: Only very little cytotoxicity of NPs at doses ~50μg/ml

LA4: ATII cells
MH-S: alv. macrophages
ANA-1: BMD macrophages

NP exposure over 96h
MH-S cells were infected with MHV-68 overnight, treated with 50 μg/ml NP and incubated for another two hours. Free lytic virus was inactivated by incubation with citrate buffer (pH=3.0). After plating on indicator cells, the amount of cytopathic effect (CPE) was determined. Relative values are given, normalized to the genomic load in the infected cells.
Influence of NP on virus reactivation: experimental setup

Cells:

- S11 (latently MHV-68 infected B cell lymphoma line)
- ANA-1/MHV-68 (BMDM/macrophage line, latently MHV-68 infected)

Experimental Setup:

Exposure of latently infected cells to 50ug NP for 72h

- Determination of the amount of lytic virus in supernatant (Plaque-Assay)
- Analysis of viral gene expression by RT-PCR (ORF50 = lytic vs. ORF73 = lytic and latent)
Influence of NP on virus reactivation *in vitro*

**S11 B cells**

**ANA-1/MHV-68 macrophages**

**virus titer**

**viral gene expression**

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* = p < 0.05

*TPA* = tetradecanoylphorbol acetate

Sattler, Part Fibre Toxicol. 2017
Exposure of persistently infected cells to TiO$_2$ NP or DEP has differential effects on virus reactivation in vitro:

S11 and ANA-1/MHV-68 cells were incubated with 50 µg/ml TiO$_2$ NP or DEP and lytic virus was determined in the supernatant by plaque assay after 72 h (panels a and b). Expression of the viral genes ORF50 (specific for the lytic phase) and ORF73 (expressed during lytic and latent phase) – shown as the ratio ORF50/ORF73 (panels c and d) was analyzed by RT-PCR 72 h after NP exposure in S11 and in ANA-1/MHV-68 cells. The value in untreated cells was set as “1” and the values for cells after NP treatment were calculated relative to the control. Data shown are the means + SD from three independent experiments. Asterisks indicate a statistically significant difference to the untreated control (*: P < 0.05).
Results *in vitro* (human cells)
Virus reactivation in human cells

LCL 1 (recombinant EBV)

LCL 2 (recombinant EBV)

LCL 3, 4 and 5 (EBV wildtype)

* = p < 0.05 vs. untreated control

Sattler, Part Fibre Toxicol. 2017
Results *in vivo*
Latent infection + NP: experimental setup

- Intranasal infection with MHV-68
- Instillation of 50ug DWCNT or Ptx90
- Harvest of lung tissue for analysis

28 days

Establishment of latency

24 hours

Sattler, Part Fibre Toxicol. 2017
Latent infection + NP: histology

IHC staining for MHV-68 proteins of the lytic phase (   )

Sattler, Part Fibre Toxicol. 2017
Latent infection + NP: transcriptome-analysis

Overlap of regulated genes, between acute infection (Virus 6d) and latent virus + NP

Virus + Ptx90:

Virus + DWCNT:

Monocytes:
- Ccl2, -7
- Cd14

Sattler, Part Fibre Toxicol. 2017
Latent infection + NP: metabolome-analysis by ICR-FT MS

Compound classes

Metabolomics virus 29d (=latent infection)

- Aminoglycosides
- Nucleosides and Nucleotides
- Sugar phosphates
- Amino acids and Peptides
- Phospholipids
- Benzene-like
- Unkowns
- N-Heterocycles
- Fatty Acids

- Green circle = not changed (compared to untreated control)
- Blue / Red circle = down / up (compared to untreated control)

Sattler, Part Fibre Toxicol. 2017
Latent infection + NP: metabolome-analysis by ICR-FT MS

Metabolomics virus 28d + Ptx90 24h (latent infection plus Ptx90)

Metabolomics virus 6d (acute infection)

= not changed (compared to untreated control)
= down / up (compared to untreated control)
Summary

**Treatment of latently infected cells (murine or human) with NP...**

- ...induces expression of the viral transactivator gene (ORF50 or BZLF1)
- ...increases the amount of infectious virus or virus genomes

**Exposure of latently infected mice to NP...**

- ...boosts production of lytic virus proteins (in monocytes/macrophages)
- ...leads to a transcriptome signature with substantial similarity to acute infection
- ...creates a metabolite composition similar to the one during acute infection
Summary

Treatment of latently infected cells (murine or human) with NP...

- ...induces expression of the viral transactivator gene (ORF50 or BZLF1)
- ...increases the amount of infectious virus or virus genomes

→ Exposure of latently infected cells or animals to NP can reactivate latent virus and restore features of an acute virus infection

- ...creates a metabolite composition similar to the one during acute infection

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Sattler, Part Fibre Toxicol. 2017
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Smart Tools for Gauging Nano Hazards

CPC
Comprehensive Pneumology Center

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