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Pneumovirus Induced Lung Disease in Mice is Independent of Neutrophil Driven Inflammation

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Introduction

The human pneumovirus: Respiratory Syncytial Virus (hRSV) is the most common cause of lower respiratory tract disease (LRTD) in young children and causes considerable mortality and morbidity.

Characteristic features of hRSV-LRTD are:

•Massive neutrophil recruitment in the lungs under influence of IL8
•Viscous DNA-rich mucus plugs obstructing the airways

Neutrophils have been proven damaging during ARDS and sepsis and may play a role in the pathogenesis of pneumovirus infections. One potential damaging effector function of neutrophils is the formation of Neutrophil Extracellular Traps (NETs), which consist of expelled DNA-fibers covered with toxic granule proteins which can capture microbes but also damage host tissue.

Hypothesis

We hypothesized that neutrophils are detrimental during severe pneumovirus disease and as such, that neutrophil depletion will lead to improved clinical and histopathological outcomes.

Aim

We aim to confirm the detrimental role neutrophils play during severe pneumovirus infection in mice. This could provide new insights in the pathogenesis of pneumovirus infections and lead to anchorpoints for new treatments.

Methods

Animals

- C57Bl6 mice (female, 8wks)
- BALBc mice (female, 8wks)

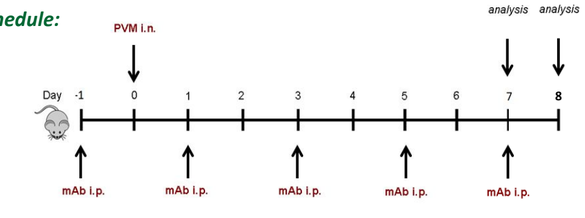
Virus & Inoculation

- Pneumonia virus of mice (PVM) strain J3666
- 2.3 x 10^4 copies of PVM intra-nasal

Neutrophil depletion

- Intraperitoneal injections with anti-Ly6G mAb (500µg, 1A8)

Schedule:



Results

Depletion efficacy

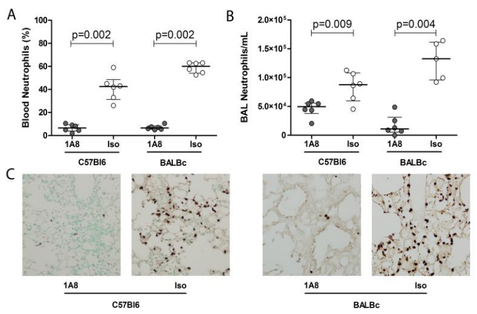


Figure 1: Significant neutrophil depletion in the 1A8 treated groups
(A) Percentage of neutrophils present in blood in C57Bl6 and BALBc mice at the final study day. **(B)** Absolute number of neutrophils per mL of BAL at the final study day. **(C)** Representative images of Ly6G-staining of lung tissue slides showing minimal interstitial neutrophil numbers in the 1A8 treated animals.

Clinical Disease

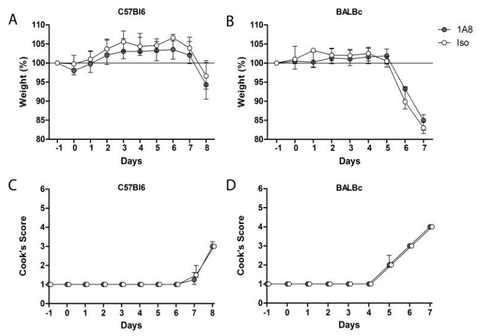


Figure 2: Neutrophil depletion does not result in attenuated disease severity
(A-B) Weight loss and clinical score of illness as measured by the modified Cook's score **(C-D)** in C57Bl6 and BALBc mice treated with either 1A8 mAb (filled dots, N=6) or isotype control antibody (open dots, N=6) during the course of severe PVM disease. No significant differences between groups. Data are shown as median with bars depicting IQR.

Semi-Survival

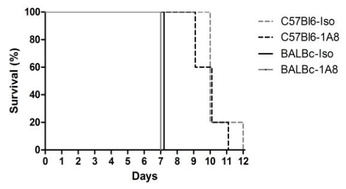


Figure 3: No increased semi-survival in the 1A8-treated groups.
Kaplan-Meier curves showing the percentages of BALBc mice (solid lines) and C57Bl6 mice (dashed lines) treated with either 1A8 mAb (black, N=5-6/group) or isotype control antibody (grey, N=5-6/group) reaching the end point of a clinical score of ≥4 and/or >20% weight loss after PVM inoculation (not significant).

Viral Load

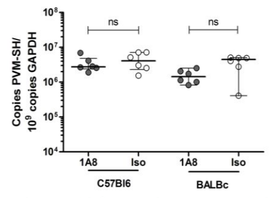


Figure 4: Neutrophils do not influence viral clearance during severe PVM infection.
Viral loads in viral copies per 109 GAPDH copies in C57Bl6 and BALBc mice, no significant differences between 1A8 mAb treated (filled dots, N=6/group) or isotype control treated animals (open dots, N=6/group).

Lung Permeability

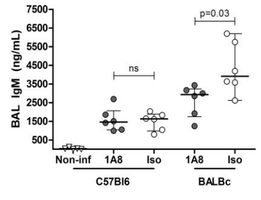


Figure 5: Neutrophils increased lung permeability during PVM infection
Lung permeability as measured by IgM (ng/mL) in BAL of C57Bl6 and BALBc mice. Increased IgM levels after PVM infection, with a significant increase in isotype control treated (open dots, N=6/group) BALBc mice, compared to 1A8 mAb treated (solid dots, N=6/group) BALBc mice (* p=0.03). Data are shown as individual values and median with bars depicting IQR.

BAL Cytokines

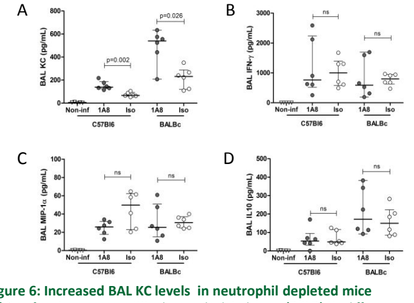


Figure 6: Increased BAL KC levels in neutrophil depleted mice
(A) Significant increases in KC values in depleted mice. **(B,C,D)** No difference in IFN-γ, MIP-1α and IL10 between groups.

Lung Pathology

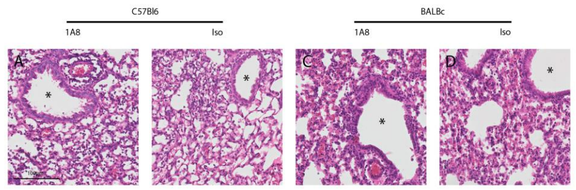


Figure 5: Neutrophil depletion does not result in altered lung histopathology
Representative image of HE-staining of C57Bl6 mice, showing alveolar cellular infiltration and proteinaceous debris, with absence of debris in the airways (asterisks) (B,D) HE-staining of BALBc mice, showing haemorrhaging and proteinaceous debris, with absence of debris in the airways (asterisks, magnification 400x).

NET formation

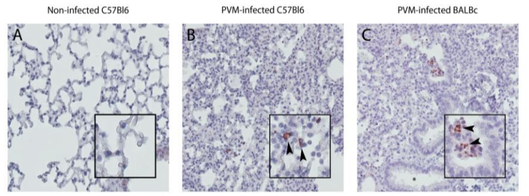


Figure 6: PVM infection does not result in significant NET production
(A) Citrullinated histone H3 staining of a non-infected lung tissue section, no NETs are visible (magnification 400x). **(B,C)** Citrullinated histone H3 staining of PVM infected C57Bl6 and BALBc mice (both isotype control treated) shows scarce NET formation (insets, magnification 800x) without airway occlusion (asterisk).

Conclusion

- ✓ **Our study shows that neutrophils do not have a major role in modulating disease outcome and viral clearance during PVM infection in mice. As such, this rodent specific pneumovirus model does not support the notion that neutrophils play a key role during severe RSV disease.**
- ✓ **Important differences in neutrophil functions between humans and mice during pneumovirus disease may exist, as shown by the relative absence of NET formation.**
- ✓ **Future studies in humans and possibly other animal models must extend these findings and further address the role of neutrophils in human RSV disease.**

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Disclosures

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