Modulation of mucosal immunity and tissue resident memory formation by NS1-deficient influenza A virus

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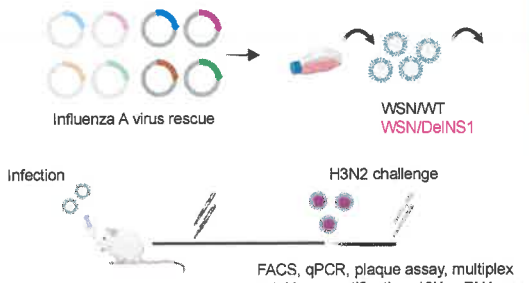
BACKGROUND

Recurrent influenza A virus (IAV) epidemics and occasional pandemics as a result of viral genome reassortment and human adaptation pose significant health burdens globally. The non-structural protein 1 (NS1) mediates attenuation of host response and facilitates viral replication in the nucleus. While intranasal vaccination using NS1-deleted live attenuated virus has been shown to induce pulmonary mucosal immunity in animal models, the immunological details have not been fully studied.

METHODS



RESULTS

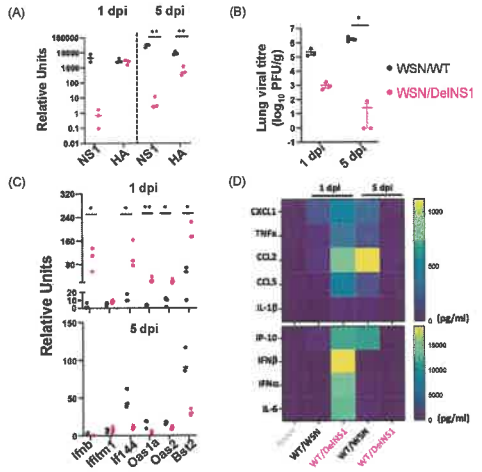


Fig. 1 Upregulation of type I IFN response and innate cytokines after infection with DelNS1 virus (A-D) Balb/c mice (6-8wk) were intranasally infected with WSN/WT or WSN/DelNS1 and subsequently sacrificed on 1 dpi and 5 dpi. Viral growth was evaluated by qPCR of NS1 and HA genes (A) and plaque assay (B). (C) Expression of type I interferon response (IFN-I) genes was evaluated by qPCR. (D) Concentrations of IFN-I and innate cytokines in bronchoalveolar lavage fluids were quantified by multiplex assay. Data in each graph represent mean ± SEM. Each experiment had 3 mice. * and ** indicate significance at *p*-values of <0.05 and <0.01 respectively.

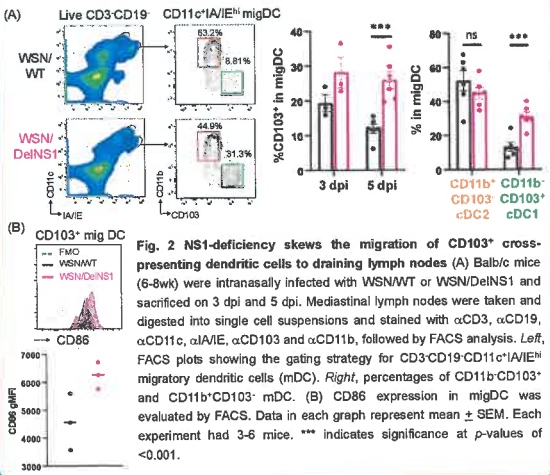


Fig. 2 NS1-deficiency skews the migration of CD103⁺ cross-presenting dendritic cells to draining lymph nodes (A) Balb/c mice (6-8wk) were intranasally infected with WSN/WT or WSN/DelNS1 and sacrificed on 3 dpi and 5 dpi. Mediastinal lymph nodes were taken and digested into single cell suspensions and stained with αCD3, αCD19, αCD11c, αIA/IE, αCD103 and αCD11b, followed by FACS analysis. *Left*, FACS plots showing the gating strategy for CD3⁻CD19⁻CD11c⁺IA/IE^hi migratory dendritic cells (mDC). *Right*, percentages of CD11b⁻CD103⁺ and CD11b⁺CD103⁻ mDC. (B) CD86 expression in migDC was evaluated by FACS. Data in each graph represent mean ± SEM. Each experiment had 3-6 mice. *** indicates significance at *p*-values of <0.001.

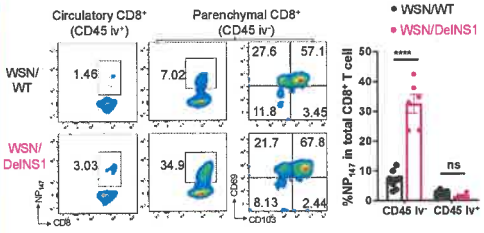
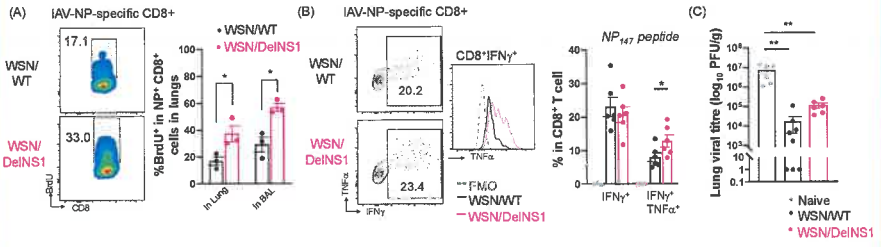


Fig. 3 NS1-deficiency enhances generation of tissue-associated influenza NP_{147}-specific T_{RM} in the lung parenchyma
Balb/c mice (6-8wk) were intranasally infected with WSN/WT or WSN/DelNS1 in the memory phase (30-35 dpi). To distinguish nonvascular cells (CD45 iv⁻) from circulatory cells (CD45 iv⁺) in the lungs, mice were intravascularly injected via tail vein with 1.5mg fluorescence-labelled αCD45 5min before sacrifice. Lung lobes were harvested and digested into single cell suspensions and stained with αCD8, αCD69, αCD103 and NP_{147} tetramer followed by FACS analysis. Data in each graph represent mean ± SEM. Each experiment had 3-6 mice. **** indicates significance at *p*-values of <0.001.



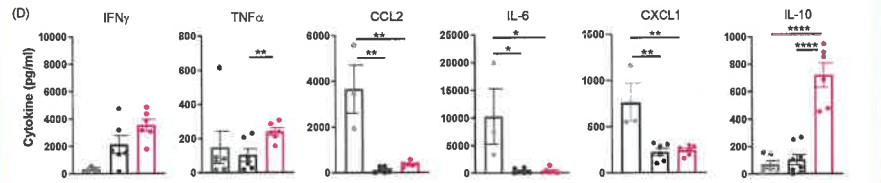
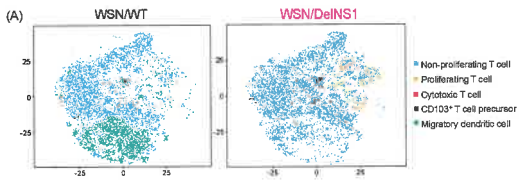
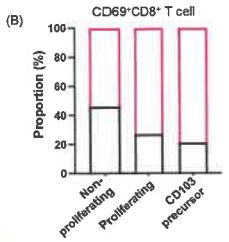


Fig. 4 Heightened activation of $CD8^+T_{RM}$ in DelNS1-immunized mice upon heterosubtypic challenge Naïve balb/c mice and mice pre-infected with WSN/WT or WSN/DelNS1 5 weeks previously were rechallenged with a lethal dose of H3N2. Five days later lungs were harvested for flow cytometry analysis. (A) Mice were intraperitoneally injected with BrdU 1 day before sacrifice. Single cell suspensions from lungs were intracellularly stained with αBrdU. FACS plots show the percentages of BrdU⁺ cells in NP_{147} tetramer-binding $CD8^+$ T cells. (B) Lung cells were cultured with NP_{147} peptide and brefeldin-A for 18h. The percentage of $CD8^+$ T cells that produced IFNγ and TNFα was quantified by intracellular cytokine staining. (C) Lungs were homogenized and viral titre was evaluated by plaque assay. (D) Concentrations of cytokines in bronchoalveolar lavage fluids were quantified by multiplex assay. Data in each graph represent mean ± SEM. Each experiment had 3-6 mice. *, **,***, **** indicate significance at *p*-values of <0.05, <0.01, <0.001 and <0.0001, respectively.





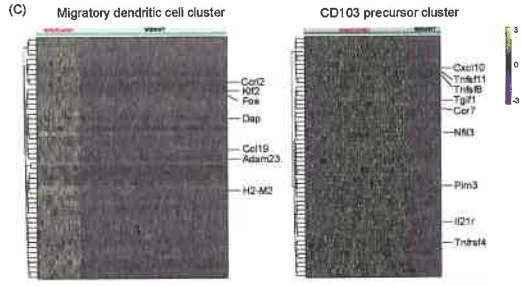


Fig. 5 Single cell RNA-sequencing reveals differential priming events in the lung draining lymph nodes after infection with WSN/DelNS1 Lung draining lymph nodes were obtained on 5 dpi with WSN/WT or WSN/DelNS1. $CD69^+CD8^+$ T cells and migratory dendritic cells were FACS-sorted for scRNA-seq. (A) tSNE plots showing the different cell clusters. (B) Percentages of non-proliferating, proliferating and CD103 precursor $CD8^+$ T cells. (C) Heatmaps showing differentially expressed genes (DEGs) in migratory dendritic cells (*left*) and CD103 precursor T cells (*right*).

CONCLUSIONS

- DelNS1 virus is replication-deficient *in vivo*, but induces massive IFN-I and innate cytokine responses.
- The unique pulmonary inflammatory environment induced by DelNS1 virus skews the preferential migration of $CD103^+$ dendritic cells into draining lymph nodes.
- DelNS1 virus leads to significant generation and deposition of lung tissue-resident $CD8^+$ memory T cells.
- $CD8^+$ memory T cells in the lungs expand massively upon heterologous influenza challenge, resulting in controlled viral replication and suppressed pulmonary inflammation.
- ScRNA-seq reveals differential priming events induced by WSN/WT *vs* WSN/DelNS1 in the lung draining lymph nodes; NS-1 deficiency leads to elevated activation states in $CD8^+$ T cells.

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