Pathogen-Mimetic Nanovaccine Protects Against Lethal Influenza Virus Infection

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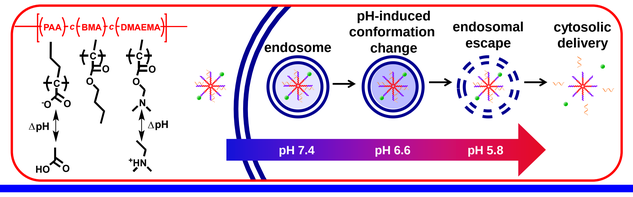
Background

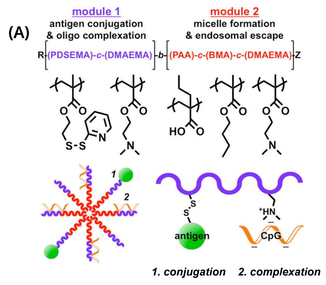
Vaccination at mucosal surfaces (e.g., lungs) with pathogen-like nanoparticles mimics natural infection and can generate tissue-resident memory T cells (T_{RM}), which are ideally positioned to trigger a protective immune response against subsequent pathogen encounter. Pulmonary immunization with subunit vaccines is an attractive approach because these are safer than vaccines based on live or attenuated microbes; they are less likely to create inflammation and damage in the lung tissue and cannot revert to a virulent form. However, they are also less immunogenic than live or attenuated vaccines, and are inefficient at generating $CD8^+$ T cells, which are necessary for defense against many intracellular pathogens, including viruses.

To address this, we have developed a pH-responsive nanoparticle (NP) delivery platform that can be loaded with protein antigen and nucleic acid adjuvant.[1] The small size of the particle (~20-40 nm) and its dual-loading capacity allows it to mimic viruses in the way it delivers cargo intracellularly and stimulates the immune system. The particle leverages endosomal acidification after cellular uptake to release antigen into the cytosol, where it can be processed by the MHC-I presentation pathway, resulting in a $CD8^+$ T cell response and lung-resident memory T cells. Here, we show this system can be used to protect mice against lethal influenza virus infection.[2]



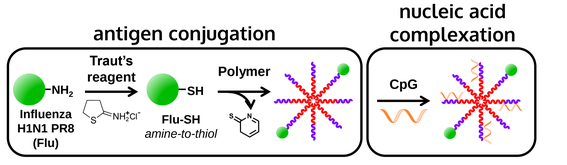
Results

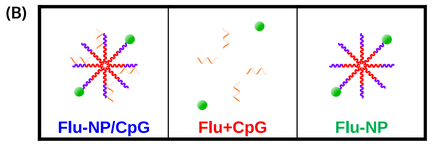
Formulating Pathogen-Mimetic Nanoparticle Vaccines

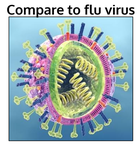


"Flu-NP/CpG"
indicates covalent conjugation
indicates electrostatic complexation
"Flu+CpG"
"+" indicates physical mixture of soluble components

"OVA"
Model antigen used to validate NP system prior to Flu studies







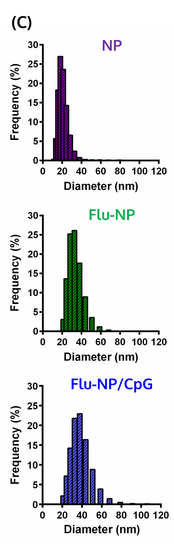


Figure 1. *pH-responsive polymer is synthesized by RAFT and loaded with protein and nucleic acid for dual-delivery of antigen and adjuvant.* ***(A)*** *Each block of the polymer corresponds to a specific function. Protein and nucleic acid can be loaded on the block that forms the micelle corona.* ***(B)*** *Schematic showing experimental and control groups used in subsequent experiments.* ***(C)*** *Dynamic light scattering (DLS) shows formation of ~20 nm diameter NP; loading with Flu protein and CpG increases the diameter to ~40 nm.*

Results

Pulmonary Immunization with the Nanoparticle Vaccine

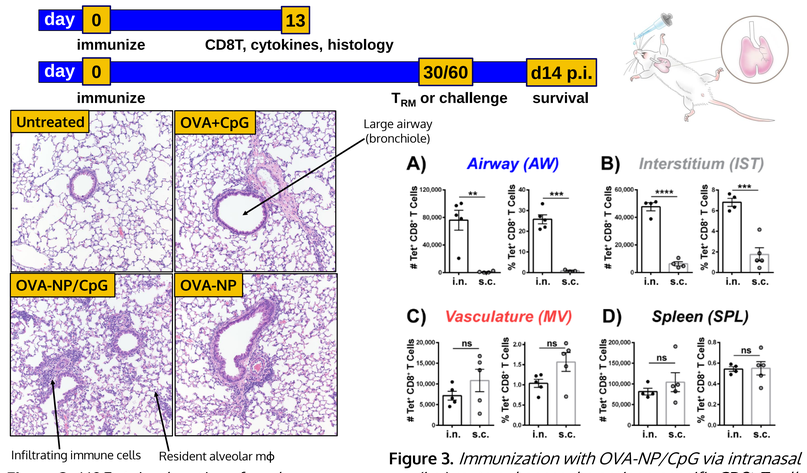


Figure 2. *H&E stained sections from lungs harvested on day 12 after immunization.*

Figure 3. *Immunization with OVA-NP/CpG via intranasal (i.n.) route enhances the antigen-specific $CD8^+$ T cell response in lungs over subcutaneous immunization.*

Lung-Resident APCs Take Up the Nanoparticle Vaccine

Figure 4. *NP are endocytosed by pulmonary antigen-presenting cells.* ***(A)*** *Fluorescence microscopy image of a lung section 24 h after immunization;* ***(B)*** *Whole lung fluorescent imaging shows differing uptake kinetics between OVA-NP/CpG and OVA+CpG;* ***(C)*** *Alveolar/interstitial macrophages and $CD103^+$/$CD11b^+$ dendritic cells took up more OVA-NP/CpG than OVA+CpG at 72 h. The NP also enhanced CD86 expression at 72 h.*

Dual-Delivery & pH-Responsive Activity Enhance Antigen-Specific $CD8^+$ T Cell Responses and Cytokine Production

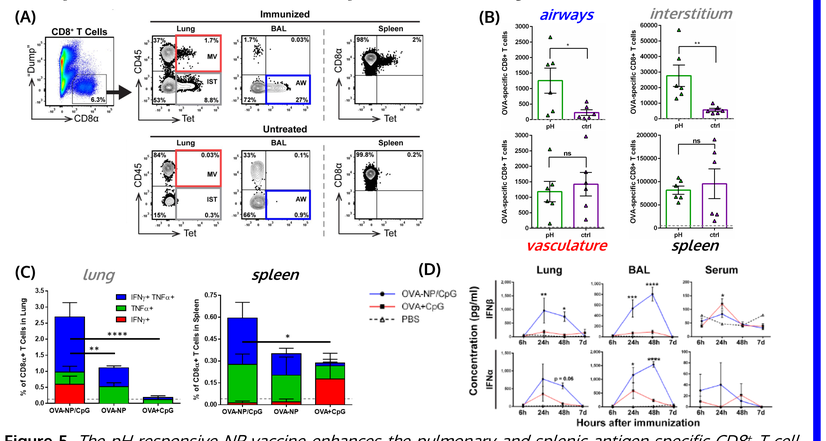


Figure 5. *The pH-responsive NP vaccine enhances the pulmonary and splenic antigen-specific $CD8^+$ T cell responses and generates polyfunctional ($IFN\gamma^+TNF\alpha^+$) $CD8^+$ T cells by d13 post-immunization, as well as T_{RM}-related cytokines at acute timepoints. (OVA dose = 7.5 μg).* ***(A)*** *Representative flow cytometry plots show how cells in lung compartments and spleen are identified;* ***(B)*** *Decreased $CD8^+$ T cell response from OVA-NP in airways and interstitium when using control polymer (ctrl) relative to pH-responsive polymer (pH);* ***(C)*** *Cytokine-producing $CD8^+$ T cells in lung and spleen;* ***(D)*** *The type I interferon response is transient, localized to the lungs and airway (BAL), and higher after immunization with OVA-NP/CpG vs. OVA+CpG. (*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ****p ≤ 0.0001)*

Results

Long-Lasting Pulmonary $CD8^+$ T Cells Express Tissue-Resident Memory Markers & Protect Against Influenza Infection

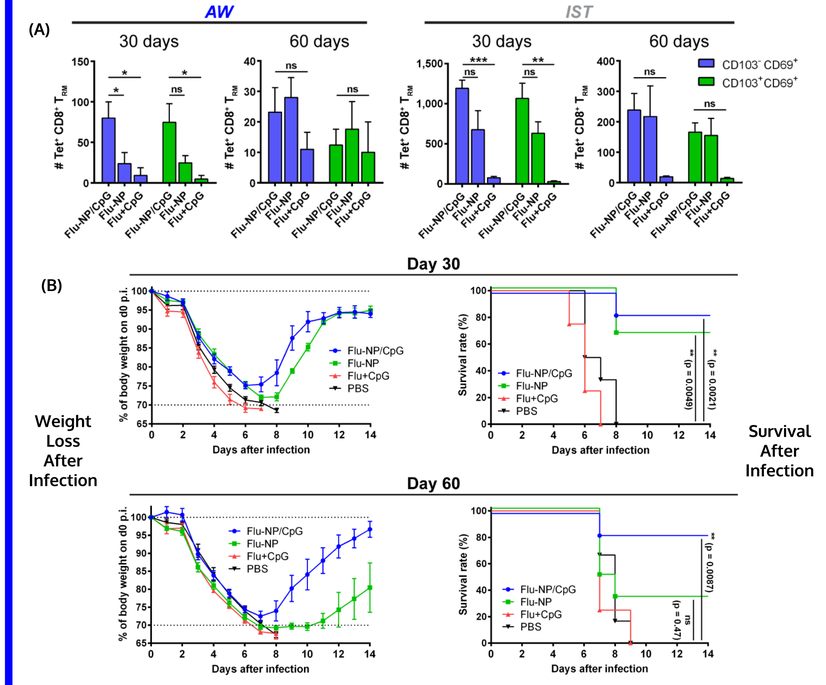


Figure 6. *Flu-specific $CD8^+$ T cells remain in the lungs for up to 60 days after immunization. We hypothesize that these are T_{RM} and contribute to protection against infection with influenza virus.* ***(A)*** *Immunization with NP vaccine enhances expression of airway- and lung-resident T_{RM} surface markers CD103 and CD69 at 30 days and 60 days post-immunization;* ***(B)*** *Intranasal challenge with a lethal dose of influenza A H1N1 virus (strain PR8) caused significantly more weight loss, greater morbidity, and and higher mortality by day 14 p.i. in mice not immunized with OVA-NP/CpG. (**p ≤ 0.01; ***p ≤ 0.001; ****p ≤ 0.0001)*

Conclusions

→ RAFT-synthesized pH-responsive polymer self-assembles to form micelles that can be loaded with influenza protein antigen and nucleic acid adjuvant

→ Intranasal administration in B6 mice causes minimal weight loss, minor local inflammation of lung tissue, and is more effective than the subcutaneous route

→ Formulation is taken up by pulmonary antigen-presenting cells, and a single dose significantly enhances the pulmonary immune response in 2 weeks

→ Antigen-specific CD8 T cells are polyfunctional and long-lasting, express lung T_{RM} markers, and protect against intranasal infection with a lethal dose of influenza A H1N1 virus for up to 60 days

→ This work has implications for development of T_{RM}-targeted vaccines against respiratory infections, as well as immunotherapies for mucosal cancers

References & Acknowledgements

[1] Wilson JT, Keller S, Manganiello MJ, *et al.* (2013). pH-Responsive Nanoparticle Vaccines for Dual-Delivery of Antigens and Immunostimulatory Oligonucleotides. *ACS Nano,* 7(5):3912-3925.

[2] Knight FC, Gilchuk P, Kumar A, *et al.* (2019). Mucosal Immunization with a pH-Responsive Nanoparticle Vaccine Induces Protective $CD8^+$ Lung-Resident Memory T Cells. *ACS Nano*, 13(10):10939-10960.

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