CRITICAL ROLE OF NASAL TISSUE-RESIDENT MEMORY CD4⁺ TH17 CELLS IN VACCINE-INDUCED PROTECTION AGAINST PNEUMOCOCCAL COLONIZATION

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Introduction

Pneumococcal Disease: Current vaccine strategies and challenges

- In 2015, lower respiratory tract (LRT) infections were the leading infectious cause of death and pneumococcal pneumonia caused over 50% of LRT deaths¹
- Most susceptible populations are infants and the elderly, particularly in developing countries
- Protein-conjugated polysaccharide vaccines protect infants but are difficult to manufacture, are expensive, require multiple injections before establishment of effective immunity, and do not include many of the capsular serotypes causing disease in the developing world
- An affordable vaccine that confers long-lasting broad, serotype-independent protection against pneumococcal disease in infancy remains a major global health priority
- Ultimate vaccine design goal: develop a vaccine that halts pneumococcus at the first step of pathogenesis which is colonization of the nasal passage and nasopharynx**

WCV elicits CD4⁺ Th17 cells that protect against nasal challenge

Figure 1: Pneumococcal Whole Cell Vaccine (WCV) immunized mice A) deficient in T-cells, or deficient specifically in CD4⁺ T cells B) treated with a CD4-depleting antibody during infection C) lacking the IL-17A receptor D) or treated with neutrophil depleting antibody, are not protected from colonization.

Background: Tissue resident memory CD4⁺ T cells (Trm) provide protection against pathogens at mucosal surfaces

Figure 2: A) Protective role of memory CD4⁺ T cell subsets B) Adoptive transfer of splenocytes and cervical lymph node cells from WCV immunized mice into Rag1^{-/-} mice does not provide significant protection to recipient mice against nasal colonization with pneumococcus C) Despite detection of high levels of WCV-induced IL-17A in the cervical lymph nodes of recipient mice D) Mice immunized subcutaneously (SQ) with WCV remain protected from nasal colonization when nasally challenged 9 months after their last immunization, indicating that **WCV provides long-lasting protection at mucosal surfaces.**

Hypothesis: Parenteral immunization with WCV generates nasal CD4⁺ Trms that provide protection against pneumococcal nasal colonization

Results

The majority of nasal CD4⁺ T cells exhibit a memory phenotype, are from an extravascular source, and exhibit a Trm phenotype

Figure 3: Characterization of CD4⁺ T cells from Nasal Lamina Propria (NLP), Cervical Lymph Node (CLN), lung or blood in mice 1 month after SQ vaccination with adjuvant alone (alum) or WCV; A) Contour plots of frequency of CD4⁺ T cells with memory phenotype (CD44^{hi} CD62L^{lo}) B) Quantitation of memory CD4⁺ T cells (Tm) from A. C) Contour plots of frequency of Thy1.2⁺ CD4⁺ T cells in NLP, CLN, lung and blood. D) Quantitation of C. E) Absolute numbers of Tm cells in NLP. F) Number of CD4⁺ cells counted in formalin-fixed paraffin embedded nasal tissue stained with anti-CD4 antibody by immunohistochemistry, n = 3-4/group. G) Histogram of expression of tissue residency-associated CD69 (tissue retention signal) and CD11a (integrin for homing to respiratory tract) on Tm cells from WCV immunized mice. H) Quantitation of Median Fluorescence Intensity (MFI) of CD69 (left) and CD11a (right) expression on Tm cells.

Pneumococcal responsive IL-17A⁺CD4⁺ T cells from an extravascular source are detected in the nose after parenteral immunization

Figure 4: NLP and CLN of SQ vaccinated mice; A) Dot plots of frequency of IL-17A⁺ or IFNγ⁺ CD4⁺ T cells post ex vivo stimulation with PMA/ionomycin or whole cell antigen (WCA) B) Frequency of IFNγ⁺ and C) IL-17A⁺ CD4⁺ T cells from A. D) Absolute numbers of IL-17A⁺ CD4⁺ T cells in NLP after ex vivo stimulation with WCA. E) Frequency of Thy1.2 IL-17A⁺ CD4⁺ T cells in NLP, CLN or lung of WCV-immunized mice post ex vivo stimulation with WCA.

WCV-induced nasal CD4⁺ Trms are sufficient to protect mice against nasal challenge, even after a single immunization as neonates

Figure 5: One month after the 3rd immunization with WCV or adjuvant alone, mice were treated with FTY720 or CD4 depleting antibody (WCV immunized mice), challenged intranasally with pneumococcus serotype 6B, and sacrificed 10 days post infection (DPI). A) Levels of nasal colonization and B) Detection of IL-17A in whole blood 6 days post ex vivo stimulation with WCA were evaluated by ELISA. C) Once month after a single immunization of six day old neonate mice with WCV or adjuvant alone, mice were treated with FTY720, challenged intranasally with pneumococcus serotype 6B, and sacrificed 10 days DPI.

WCV-induced nasal CD4⁺ Trms are sufficient to mediate recruitment of neutrophils to the nose after nasal challenge

Figure 6: After performing nasal washes on mice from figure 5, nasal tissue was collected, formalin-fixed and paraffin embedded. Tissue sections were stained with anti-CD4 (T cell) and anti-MPO (neutrophil) antibodies and imaged by confocal microscopy. A) Example of images of CD4⁺ and MPO⁺ cells in nasal passage. Top four images = 40X tile of maxilloturbinate (MT) in T1-T2 level of nasal passage. Bottom image = 40X zoom of same MT from FTY720 treated mouse. Quantitation of CD4⁺ cells B + D, MPO⁺ cells C + E, n = 5-8/group. For mice immunized as neonates, the number of CD4⁺ and MPO⁺ cells were determined for FTY720-treated mice only.

Conclusions and Future Studies

- Parenteral vaccination with WCV induces nasal IL-17A-producing CD4⁺ Trms that protect against nasopharyngeal colonization
- Vaccine-induced CD4⁺ Trms protect against nasal challenge by facilitating IL-17A-mediated neutrophil recruitment to the nose
- Measurement of nasal CD4⁺ Trm IL-17A responses might be a better indicator of WCV (or other protein antigen) efficacy than splenic or whole blood IL-17A responses
- Understanding how these Trms are generated may have implications for next generation vaccine design
- To understand how CD4⁺ Trms are generated in the nose after SQ immunization, we will transfer CD45.2⁺ OTII T cells into CD45.1⁺ congenic mice and immunize with OVA-expressing WCV, and track the development of OVA-specific CD4⁺ T cells in nose
- To determine if induction of nasal Trm is unique to WCV, we will investigate the generation of antigen-specific Trms in the nose after parenteral vaccination with OVA, nonpathogenic streptococci, other respiratory pathogen e.g. Influenza virus, enteric pathogens e.g. *Salmonella typhimurium*.

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