**Influenza in Phagocytic Cells: Direct Infection or Exogenous Antigen Uptake**

Thomas Schmidt MHS, Jessica K. Fiege PhD, and Ryan A. Langlois PhD
University of Minnesota, Department of Microbiology and Immunology, Center for Immunology

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**Abstract**

Influenza A virus (IAV), is an eight segmented, negative sense RNA virus. IAV is an extremely infectious virus that infects 9.2-35.6 million people in the US each year. Understanding the viral kinetics in the host is critical to developing an effective vaccination strategy. Phagocytic cells such as macrophages and dendritic cells (DCs) are a vital link between the innate and adaptive immune systems, trafficking antigen from the site of infection to the draining lymph node (dLN) and presenting antigen to T cells. While phagocytic cells have been reported to contain IAV antigen, it is unclear if phagocytic cells obtain IAV antigen either through direct infection or phagocytosis. We developed an IAV expressing Cre recombinase (IAV_Cre) to specifically label infected cells in a Cre-inducible tdTomato (iRED) reporter mouse. Cre recombinase selectively removes a loxp flanked stop cassette and allows for the subsequent transcription of the fluorescent reporter tdTomato. This genetic alteration is irreversible and indelible, allowing us to track any cell that has ever been infected. Previous studies have characterized tdTomato expression in epithelial cells in the lung after IAV infection, while immune cells have not been studied in this system. While dendritic cells (DCs) and macrophages (MΦs) have been reported to be infected by IAV, it is unclear in vivo how many of these cells are actively infected, or have phagocytosed IAV antigen. Previous studies have shown a minority of CD45+ cells positive for IAV infection; we hypothesize that the majority of reporter+ DCs and MΦs have phagocytosed antigen opposed to being directly infected. We sought to 1) identify what subsets of phagocytic cells are reporter+ at various time points after IAV_Cre infection, and 2) determine if phagocytic cells are directly infected or take up exogenous tdTomato after IAV Cre infection of iRED mice. To determine the peak of tdTomato+ phagocytic cells, we harvested lungs, dLNs, and spleens on days 3, 5, 7, 10, and 21 post infection (dpi). We observed reporter+ phagocytic cells in the lung and dLNs at multiple time points after infection. Loss of reporter+ cells in the lung corresponded with a concurrent gain of reporter+ phagocytic cells in the dLNs over the time course. To directly assess if phagocytic immune cells can obtain tdTomato via phagocytosis, we used a bone marrow derived MΦs culture system. We were able to demonstrate that MΦs can successfully phagocytose tdTomato from IAV_Cre infected iRED fibroblasts. Using the bone marrow chimera mice, we were able to demonstrate MΦs and DCs can phagocytose tdTomato in vivo. These data demonstrate that phagocytic immune cells can phagocytose IAV antigen and traffic to secondary lymphoid organs. These results have implications to IAV vaccination demonstrating that subunit vaccinations are a viable option for priming adaptive immune cells in secondary lymphoid organs.

**Project Aims**

- Determine if MΦs phagocytose take up exogenous fluorophore.
- Determine kinetics of reporter+ MΦs and DCs.
- Determine if reporter+ MΦs and DCs are reporter+ due to direct infection or exogenous uptake of fluorophore.

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**Figure 1: IAV_Cre permanently labels infected cells**

**Figure 2: MΦs phagocytose tdTomato in vitro**

**Figure 3: Reporter+ cells are present in lung and draining lymph nodes**

**Figure 4: Phagocytic cells phagocytose tdTomato in vivo**

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**Conclusions**

- BMDM can phagocytose tdTomato fluorophore in vitro and in vivo
- Reporter+ MΦs and DCs are at the greatest abundance at 5 days post infection (dpi)
- Reporter+ MΦs and DCs are barely detected past 10 dpi
- Phagocytic immune cells can phagocytose IAV antigen and traffic to secondary lymphoid organs. These results have implications to IAV vaccination demonstrating that subunit vaccinations are a viable option for priming adaptive immune cells in secondary lymphoid organs.

**Future Directions**

- Replace WT bone marrow with iRED donor bone marrow to determine the frequency of reporter+ phagocytic cells due to direct IAV infection.
- Assess activation levels of CD80/86 in direct infection versus exogenous uptake of antigen.
- In exogenous uptake of tdTomato, determine if changes in tdTomato reporter+ populations are due to trafficking to and from tissues, degradation of the tdTomato fluorophore, or if phagocytic cells dying.

**References**


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