

The Role of Indoleamine 2,3-Dioxygenase in CD8 T Cells Responses to Secondary Influenza  
Infection

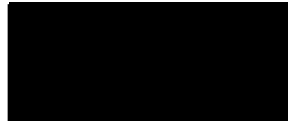
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### **Objective**

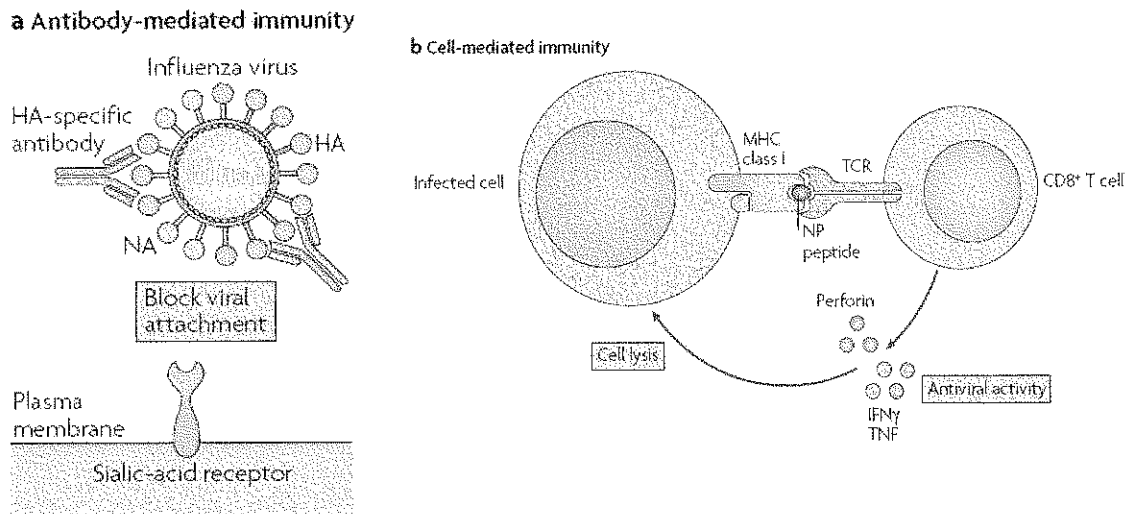
To determine whether the loss of indoleamine 2,3- dioxygenase results in a more robust influenza specific CD8<sup>+</sup> T cell response to influenza infection.

### **Abstract**

Current influenza vaccines are designed to generate antibody-specific immunity against predetermined strains of virus and are ineffective in protecting against novel or emerging strains. Targeting conserved T cell epitopes can provide protection against unexpected circulating strains of influenza, however, this protection wanes over time. Thus, understanding and exploiting the mechanisms involved in CD8<sup>+</sup> T survival and maintenance may aid in the development of a successful anti-influenza vaccine eliciting cell-mediated immunity. Indoleamine 2,3-dioxygenase (IDO) is a tryptophan catabolyzing enzyme up regulated following influenza infection, which suppresses naive T cell proliferation and induces the apoptosis of T cells. To determine whether IDO suppression may be a viable option to enhance T cell immunity to influenza infection, wild type and IDO knockout mice were infected with influenza, and the number, frequency, and function of the influenza-specific CD8<sup>+</sup> T cells were monitored over time. IDO knockout mice demonstrated a more robust virus-specific T cell response to influenza in the lung and lung airways (the site of viral replication) compared to wild-type during the early effector phase of the response. However, both groups of mice harbored similar levels of influenza-specific CD8<sup>+</sup> T cells at later stages of the immune response. Future experiments will determine whether an IDO deficiency alters the phenotype and function of these memory cells when rechallenged with influenza. Together our data provides a detailed picture of how IDO regulates T cell responses to respiratory infection.

### Introduction

Approximately twenty-five to forty million U.S citizens are affected annually by influenza, with over 150,000 individuals hospitalized and 30,000 deaths in the United States alone (Flu Facts). The immune system plays an essential role in protecting the body as one of its main roles is the clearance of viral antigens (Thibodeau et al., 2005, p. 472). Currently, every year, Americans must get vaccinated annually as the current vaccination strategy elicits an antibody-mediated response; however, this strategy in protecting the host from novel strains of influenza. Targeting conserved viral epitopes with a cell-mediated immune response can provide protection against unexpected circulating strains (Subbaro et al., 2007). Therefore, studying mechanisms to elicit the number of T cells may be a more efficient vaccine strategy.



### **Figure 1 – Immune Responses to Influenza**

(A) Antibody-mediated immunity – Antibodies specifically bind to viral glycoproteins, preventing infection of cells. (B) Cell-mediated immunity – CD8<sup>+</sup>T cells specific for viral proteins such as nucleoprotein (NP) recognize viral peptides presented by MHC class I molecules. Upon recognition, cytokines and perforins are released (Subbaro et al., 2007).

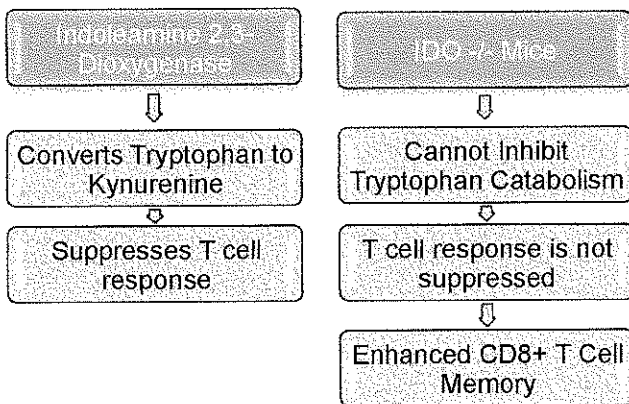
Prior to encountering a foreign antigen like a flu virus, CD8 T cells' ability to respond to a specific infection is minimal, and it cannot migrate to the site of the infection. The pathway

that the CD8 T cells migrate to is limited to the blood and lymphatic fluid, but upon encountering an antigen, T cells clonally expand, peaking at approximately ten days post infection (Abbas et al., 2010, p. 10). The CD8+ T cells become activated, and some of their properties change, such as its function and range. The activated CD8+ T cells have the ability to migrate to non-lymphatic tissue such as the lung airway and lungs in the case of a mucosal infection like influenza.

After lymphocyte activation and clearance of antigens, the T cell population contracts, leading to a memory population, which essentially remembers the encountered pathogen. Upon encountering a similar antigen that shares the same viral epitopes, the memory T cell population expands faster and much more strongly than naïve T cell. According to a model regarding the T-cell system, there are two classes of memory T-cells: central memory and effector memory T cells. Central memory and effector memory have distinct roles. Effector memory T cells migrate to peripheral tissues and have an effector function, but central memory T cells have little effector function but can readily expand (Sallusto et al., 2004).

Indoleamine 2,3-dioxygenase (IDO) is a tryptophan-catabolyzing enzyme that has been found to be one of the mechanisms that contributes to tumor-induced tolerance by inhibiting the immune system through the local depletion of tryptophan (Munn et al., 2007)., IDO suppresses the immune system inhibition T cell proliferation and activation of regulatory T cells (Mellor et al., 2002). Although recent work has elucidated the relationship between IDO and immunosuppression, there has been little work done on the effects of tryptophan depletion on the ability of CD8 T cells to respond to influenza. IDO expression is induced by influenza infection in the trachea, lung, spleen, and pancreas beginning around day 5 p.i., peaking around day 11 p.i, and dropping around day 14 p.i (Yoshida et al., 1979).

In order to effectively study the relationship between IDO and memory T-cell response, genetically engineered IDO knockout mice (IDO  $-/-$ ) were used to determine whether the loss of indoleamine 2,3- dioxygenase results in a more robust influenza specific CD8<sup>+</sup> T cell response to influenza infection. Therefore, we hypothesized that the loss of IDO would result in a more robust CD8<sup>+</sup> T cell response to influenza infection.



### Figure 2 - Hypothesis

IDO enzyme activity leads to immunosuppression through activation of T<sub>regs</sub> and prevention of proliferation. Consequently, the IDO  $-/-$  should not be able to suppress the proliferation of T cells and thus should have an enhanced memory CD8<sup>+</sup>T population.

## Materials and Methods

### *Infection of Mice*

Sex matched C57BL/6 and C57BL/6 IDO  $-/-$  were infected with  $10^3$  PFU HKx31 influenza virus. After 84 days post infection, mice were reinfected with a lethal challenge of  $5 \times 10^3$  PFU PR8 influenza virus. Mice were weighed daily. Mice were taken down on day 10 and lungs, spleen, and lymph nodes were extracted from mice and processed.

### *Flow Staining*

Single-cell suspensions were obtained from the spleen, mediastinal lymph node, and peripheral lymph node by passing homogenized organs through cell strainers followed by depletions of erythrocytes. Bronchoalveolar lavage (BAL) cells were harvested through

introduction and recovery of 1 mL of PBS 3x. Lungs were perfused using PBS with heparin. Cells were placed through cell strainers and then placed in a Percoll gradient. After centrifugation, cells at the interface were collected, washed, and counted.

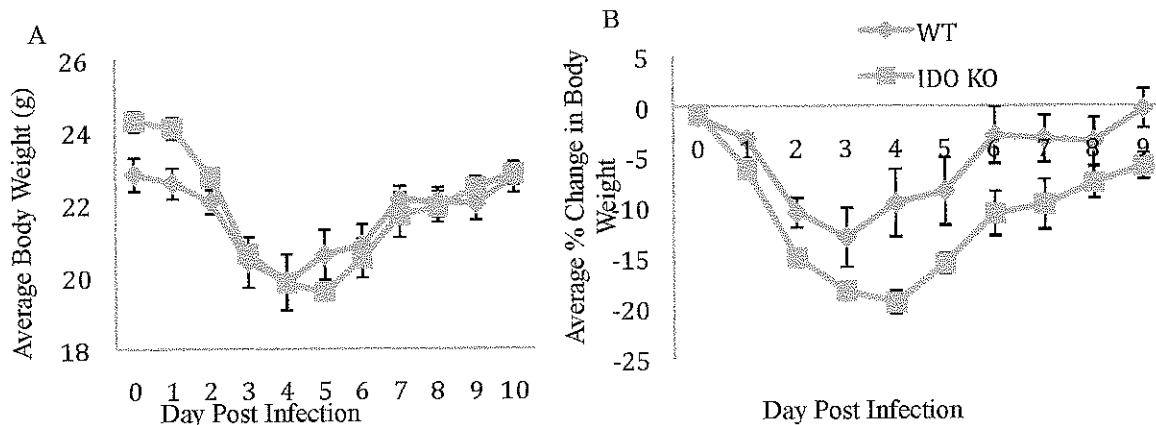
An antibody solution was prepared using fluorophores that will fluoresce at various wavelengths by a flow cytometer. Lymphocytes were analyzed on a BD LSRII for reactivity with anti-CD8, anti-CD11a, and NP- loaded MHC I tetramer.

#### *Data Analysis*

The samples were analyzed using BD FACSDiva. Lymphocytes were selected, and CD8+ T cells were gated. Next, CD8+ T cells that were CD11a and NP-Tet+ were gated. The percent NP-Tet+ values were recorded and plotted using Excel.

### **Results**

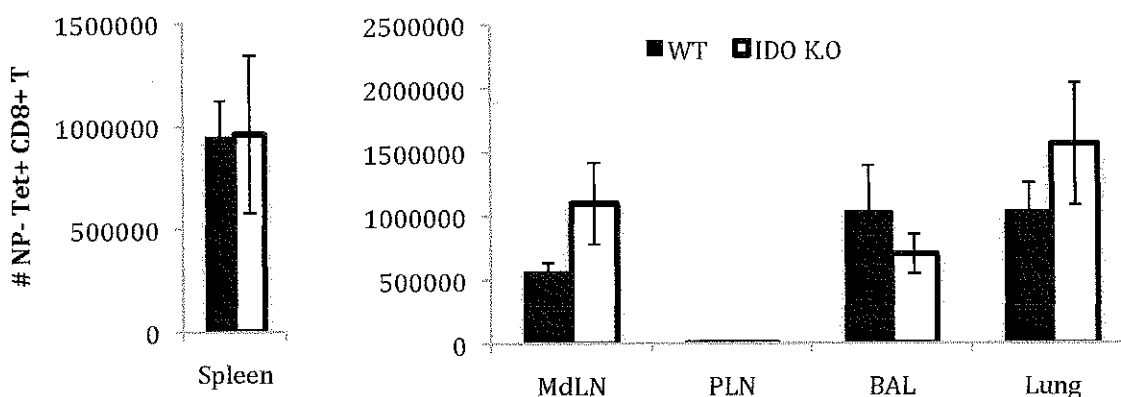
Upon reinfesting mice with a lethal challenge of PR8, mice were weighed daily. Shown in figure 3 are the average body weights of mice over a ten-day period. The average change in body weights were calculated too and shown in figure 3. IDO knockout mice have a much greater change in average body weights over the ten-day period in comparison to WT mice.



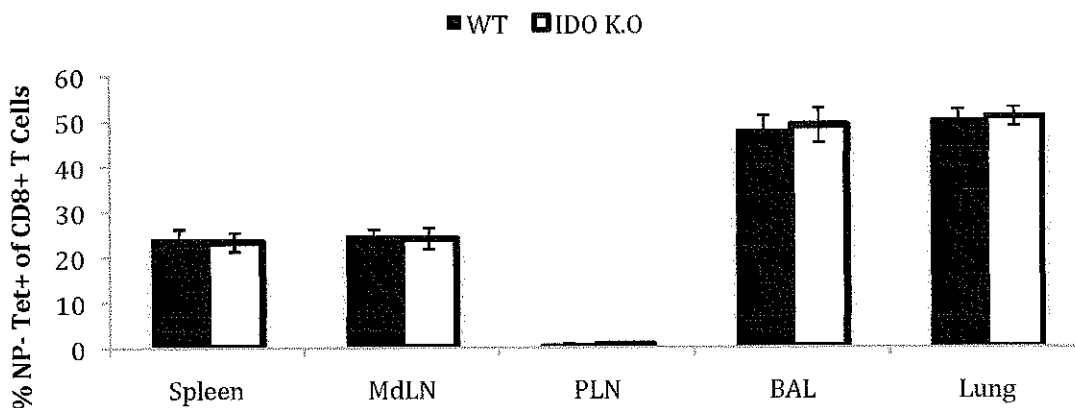
**Figure 3 - IDO deficient mice show a much larger weight loss upon PR8 influenza challenge.** Depicted are average weights (+/- SEM) for WT and IDO  $-/-$  at d10 p.i. ( $n > 6$  mice per group). (A) Average body weights. (B) Average percent change in body weights.

After ten days post-challenge, single-cell suspensions were obtained from spleens, lungs, and cells from the lung airways. IDO deficient mice show had a slightly larger number of NP-Tet+ CD8+ T cells than wild-type controls in the draining lymph node only. Interestingly, the percent NP-Tet+ of CD8+ T cells of both IDO deficient mice and WT mice were similar as shown in figure 4.

A.



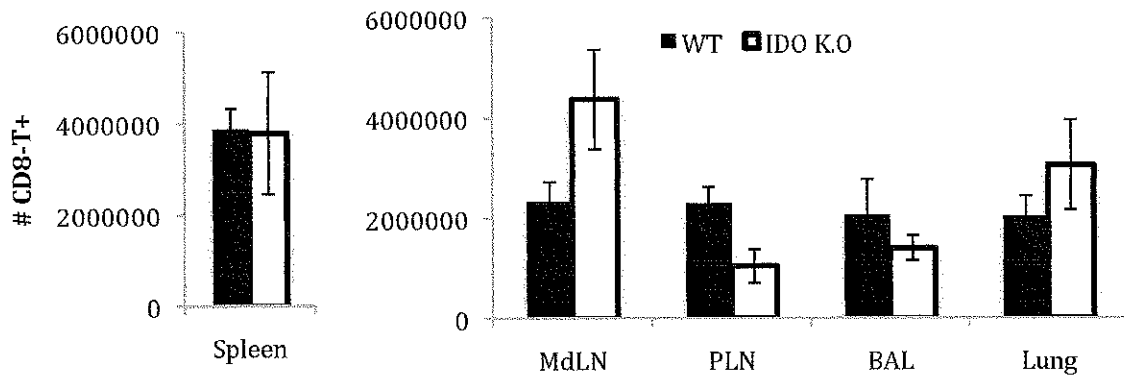
B.



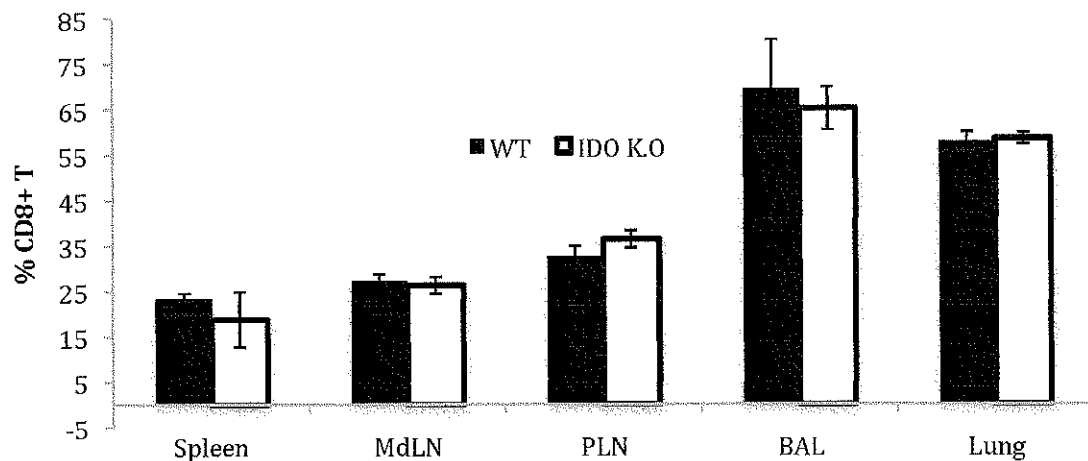
**Figure 4 - Lack of the enzyme IDO slightly increases anti-NP-specific CD8+ T cell response at day 10 post challenge.** Depicted are average NP- Tet+ total CD8s (+/- SEM) isolated from the indicated tissues for WT and IDO -/- at d10 post-challenge. (n=4 mice per group. (A) Percentage NP- Tet+ CD8+ T Cells. (B) Number of NP- Tet+ CD8+ T Cells.

In addition, the number and percentage of CD8<sup>+</sup>T cells were gated as well. IDO <sup>-/-</sup> mice do not show a significant difference in the percentage of CD8<sup>+</sup>T; however, the number of CD8<sup>+</sup>T cells were higher in IDO deficient mice than in the control WT mice only in the draining lymph node. In the lung, there is a slight increase in the number of CD8 T cells, but it is not a significant increase.

A.



B.



**Figure 5 – IDO deficiency slightly increases CD8<sup>+</sup> T cell response at day 10 post challenge.** Depicted are average total CD8s (+/- SEM) isolated from the indicated tissues for WT and IDO <sup>-/-</sup> at d10 post-challenge. (n=4 mice per group. (A) Percentage CD8<sup>+</sup> T Cells. (B) Number of CD8<sup>+</sup> T Cells.



### **Discussion**

Upon a challenge using PR8 influenza virus, IDO  $-/-$  seem to have a higher number of both CD8 $^+$ T cells and NP-Tet $^+$  CD8 $^+$ T cells in the mediastinal lymph node. Our previous data showed that during the effector phase of the CD8 T cell response, the lack of IDO lead to a higher population of NP-Tet $^+$  CD8 $^+$  T cells in the lung airways, lung, and spleen, but it did not lead to a higher number in the mediastinal lymph node. However, during the lethal challenge with PR8, this trend did not hold. Interestingly, during the challenge, the mediastinal lymph nodes was the only tissue studied in which there was an enhanced CD8 $^+$  T cell response.

IDO has been shown to suppress the immune response. Interestingly, although it has been previously shown that levels of IDO are induced in the pancreas and spleen upon infection with influenza, only the draining lymph node seems to a decrease in the number of T cells generated. Presently, the experiment only shows the number of CD8 $^+$  T cells and NP-Tet $^+$  CD8 $^+$ T cells. The next series of experiments will compare the functionality of the anti-influenza memory CD8 $^+$  T cells generated in WT and IDO  $-/-$  mice. The effects of IDO on the function of CD8 $^+$  T cells will be studied by using plaque assays, which assess the ability of CD8 $^+$  T cells to clear virus. Preliminary data shows that T cells from an IDO deficient mice clear flu virus at the same rate as WT control mice.

## References

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