

## **HIV-1 elicits a pDC dependent IFN- $\alpha$ response in the human thymus**

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Interferon alpha (IFN- $\alpha$ ) is a potent modulator of immune responses. Its primary function is to prevent the spread of infection through the upregulation of interferon response genes, like MxA in humans. While any white blood cell in the body can produce IFN- $\alpha$ , it has been reported to be secreted at higher levels by plasmacytoid dendritic cells (pDC). Production and secretion of IFN- $\alpha$  by pDC occurs in response to viral (HSV, Influenza, Sendai, HIV) and bacterial infections (CpG ODN, single-stranded RNA). We have previously shown that pDC are essential for triggering upregulation of MxA in thymocytes. The aim of this study is to further elucidate the breadth and strength of IFN- $\alpha$  responses during HIV-1 infection in the human thymus.

Intracellular flow cytometry for MxA combined with cell surface staining was used to identify interferon responding cells in mock infected or HIV-1 infected thy/liv implants from SCID-hu mice. We observed that in the absence of HIV-1 infection pDC constitutively express MxA. MxA expression was not isolated to the pDC population as it was also found within some CD3 positive thymocytes. Following HIV-1 infection almost 90 percent of all cells expressed MxA.

In addition, we were able to show that the majority of productively infected thymocytes upregulate MxA by dual intracellular stain for HIV-1 p24 and MxA. However, the majority of MxA positive cells do not express HIV-1 proteins, suggesting that the virus induces a bystander IFN- $\alpha$  response in the thymus. Comparison of the level of MxA expression within the p24 positive and the p24 negative subsets showed that productively infected cells express more MxA than their uninfected counterparts. By examining the ability of HIV-1 to induce an IFN- $\alpha$  response *in vitro* we found that productive infection was necessary to upregulate MxA expression. Cultures that were infected with HIV-1 for less than two days (no detectable p24) did not differ in their MxA expression from cultures that were infected with mock supernatant.

Our previous studies and those by others have demonstrated the ability of IFN- $\alpha$  to delay HIV-1 replication in the thymus. Our current data confirm the presence of IFN- $\alpha$  in response to HIV-1 by showing a robust upregulation of the interferon response gene MxA in thymocytes. However, neither the IFN- $\alpha$  produced nor the upregulation of MxA is effective in fully preventing HIV-1 replication in the thymus. This lack of control may be due to a loss or functional impairment of pDC during HIV-1 infection resulting in an overall decrease in the interferon response.