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### **► To cite this version:**

Monica Montes, Nicolas Loof, Amanda Cobb, David Jutras, Charlie Quinn, et al.. P16-49. Broad types of cytokines secreted by Gag-specific T cells from HIV infected patients on HAART. AIDS Vaccine 2009, Oct 2009, Paris, France. pp.P278, 10.1186/1742-4690-6-S3-P278 . inserm-00663921

**HAL Id: inserm-00663921**

**<https://inserm.hal.science/inserm-00663921>**

Submitted on 27 Jan 2012

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## **P16-49. Broad types of cytokines secreted by Gag-specific T cells from HIV infected patients on HAART**

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from AIDS Vaccine 2009  
Paris, France. 19–22 October 2009

Published: 22 October 2009

*Retrovirology* 2009, **6**(Suppl 3):P278 doi:10.1186/1742-4690-6-S3-P278

This abstract is available from: <http://www.retrovirology.com/content/6/S3/P278>

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### **Background**

Previously described highly immunogenic epitopes from Gag are able to stimulate Th1/CTL cells. This response is essential for controlling progression of HIV infection. Techniques like IFN- $\gamma$ -ELISPOT and IFN- $\gamma$  intracellular staining are currently the standard methods to define antigen-specific immune responses. A recent method, the Luminex analysis allows the identification of responses characteristic of T cell subtypes through the secretion of not only IFN- $\gamma$  but other key cytokines (IL-5, IL-13, IL-10, IL-21 and IL-17).

### **Methods**

We have focused on defining the appropriate conditions to study antigen-specific T cell memory responses in chronically infected HIV patients. Briefly, short-term PBMC cultures with 15-mer overlapping peptides from Gag in the presence of IL-2 favor expansion of antigen-specific T cells. Luminex analysis of the culture supernatants, permits simultaneous identification of cytokine profiles. HIV-specific clones obtained with selected Gag peptides were stimulated with different concentrations of antigens followed by cytokine secretion analysis.

### **Results**

We identified anti-Gag responses to already well known peptides as well as new ones consistent with each patient's HLA profile. Interestingly, this analysis revealed that some epitopes induced a complex pattern of cytokine secretion, including Th1/Th17, Th2 and IL-21 responses. CD4+ T

cell clones expressing IL-21 are able to secrete Th1 and Th2 cytokines as well. The same results were obtained with CD8+ clones; however the secretion of IL-21 is lower than in CD4+ cells. In the CD4+ and CD8+ T cell clones the responses are directed to Th1 or Th2 based on the concentration of the antigen used to stimulate the cells. However, Th17 specific cells were only able to secrete IL-17 in an antigen-specific test.

### **Conclusion**

Our approach will help monitor therapeutic vaccine responses in planned clinical trials by establishing knowledge of each patient's immune status, thereby permitting comparison between correlates of viral replication control and vaccine-elicited immune responses.