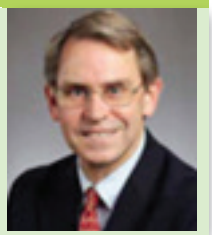
Johnson: Single cell transcriptional profiling of latently infected CD4+ T cells

OVERVIEW

HIV-1 rapidly establishes a latent infection of long-lived quiescent CD4+ T cells, which carry an integrated provirus that is largely transcriptionally silent, functionally invisible to immune surveillance and impervious to the activity of antiretroviral drugs. The long half-life of these cells and their capacity to be reactivated and produce infectious virions remain the primary obstacles to viral eradication. Currently research techniques have numerous limitations, because the relative rarity of latently infected cells severely constrains efforts to dissect molecular mechanisms involved in latency. Furthermore, the predominant reservoirs of latently infected cells are found in secondary lymphoid and mucosal tissues, not in peripheral blood, the primary site that is readily available for studies in HIV-infected human subjects. We propose a novel approach using a highly sensitive, high throughput single cell qPCR technique to analyze CD4+ T cells isolated from lymphoid tissues from SIV-infected macaques treated with potent antiretroviral therapy (ART), coupled with the identification of subsets of memory CD4+ T cells that are highly enriched for latently-infected cells. By analyzing expression of a panel of molecules that are differentially expressed on the surface of CD4+ T cells, we will identify novel cell surface biomarkers, that either alone or in combination, represent a distinct signature of latently infected cells. The use of single cell transcriptional profiling represents a unique approach to address the fundamental challenges in characterizing latently-infected reservoirs and should provide essential information that could be then used for design of therapies to selectively target latently-infected cells in HIV-infected patients.

This grant is led by R. Paul Johnson, MD (Emory University, Yerkes National Primate Research Center). The award was received in November, 2014, with an initial agreement length of 2 years.

Grant at a Glance



Principal Investigator
R. Paul Johnson, MD

Grantee Institution
Emory University, Yerkes National Primate Research Center, Atlanta, USA

Project Title
Single cell transcriptional profiling of latently infected CD4+ T cells

OPPID
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Grant Award
Up to $1.2 million, awarded in November, 2014

RESEARCH OBJECTIVES

1.) To establish potent suppression of viral replication in SIV-infected macaques using optimized ART regimens. From such treated animals, we will collect and cryopreserve a comprehensive set of tissues that includes, inter alia, secondary lymphoid and mucosal tissues, and CNS tissues

2.) To apply recent advances in the characterization of memory CD4+ T cells to identify enriched reservoirs of latent SIV-infected cells. In addition, we will include monocytic cell populations in the analysis

3.) To apply single cell transcriptional profiling to identify novel biomarkers present on the surface of latently infected cells. Our primary method for such profiling is real-time PCR, and we will evaluate RNAseq as an alternative means for such characterization