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Institutionen för Mikrobiologi, Tumör och Cellbiologi

Characterization of HIV-1 populations in infected cells

AKADEMISK AVHANDLING

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ABSTRACT

Human immunodeficiency virus (HIV) is one of the most fatal diseases in the world today, and the most important worldwide infectious disease in terms of mortality. This virus infects and kills cells of the immune system involved in defending the body against infectious agents such as viruses and bacteria. Infected cells can circulate throughout the body enabling HIV to disseminate and cause infection in many different anatomical compartments. HIV can either establish active infection causing the death of the infected cell or become a latent infection allowing the virus to escape HIV specific immune response and antiretroviral therapy (ART). Latent infection can keep the virus silent for years hindering the possibility of a cure with current therapies. In addition, HIV can quickly evolve into new viral variants which can escape immune pressure, develop resistance to ART and impede the development of a functional vaccine.

In order to better understand the pathogenesis and dynamics of HIV infection it is important to analyze the infected cells and the viral variants that reside within them. In this thesis, highly sensitive assays have been developed and used to analyze which cells are infected and the genetic makeup of the HIV-1 variants within these infected cells isolated from different cellular populations and anatomical compartments from treatment naïve and treatment experienced patients.

In **papers I and II** the number of HIV-1 DNA molecules in single infected cells from peripheral blood and lymph node tissue from untreated patients was evaluated. The results from these studies revealed that the majority (>90%) of the CD4⁺ T-cells from peripheral blood and lymph node tissue contained only one HIV-1 DNA molecule. This result is in contrast to the generally accepted belief that most HIV-infected cells contain multiple HIV DNA molecules. In addition, we demonstrated a similar genetic composition of HIV-1 in lymph node tissue, peripheral blood and plasma. This finding indicates an exchange of virions and/or infected cells between these compartments during untreated early and chronic HIV-1 infection.

In **papers III and IV** we analyzed the genetic makeup of HIV-1 populations located in different infected cells from patients on long-term suppressive therapy. In **paper III** we isolated CD34⁺ hematopoietic progenitor cells (HPCs) from bone marrow to investigate if these cells are an HIV-1 reservoir in patients during long-term suppressive therapy. In conducting this study we found no HIV-1 in 100 000 to 800 000 CD34⁺ HPCs analyzed per patient indicating that CD34⁺ HPCs from the bone marrow are not a source of persistent HIV-1.

In **paper IV** we analyzed infected cells from gut associated lymphoid tissue (GALT) and peripheral blood isolated from the same patients described and studied in **paper III**. Despite several years of therapy we found a high infection frequency of cells isolated from these patients. Moreover, the infection frequency of these cells was greater in patients treated during chronic infection compared to patients treated during early infection in both peripheral blood and GALT. In addition, we evaluated the degree of HIV-1 genetic change between samples isolated before the patients started therapy and after several years of therapy to understand if on-going viral replication can maintain HIV-1 during suppressive therapy. Our results show that very little change has occurred during years of suppressive therapy in the patient samples analyzed.

In conclusion, the work presented in this thesis has provided new techniques to analyze the HIV-1 reservoir. The use of these techniques has resulted in a better understanding of which cell types act as HIV-1 reservoirs and the genetic nature of the virus residing in these infected cells during early and chronic infection in both treatment naïve and experienced patients.