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كلية الطب البيطري
قسم الفيروسات

Analysis of Granzyme B- Expressing B Cells in SIV-Infected Rhesus Macaques

Thesis presented by

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(B.V.Sc., Faculty of Vet. Med., Cairo University, 2011)

For the degree of M.V.Sc
(Virology)

Under the Supervision of

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Supervision Sheet

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Abstract:

HIV infection continues to be a major global health issue and is characterized by a profound dysregulation of various immune cells, including B cells. Recently, increased frequencies of regulatory, granzyme B-expressing B cells have been identified in HIV-infected patients compared to healthy subjects, but their function remains unclear. Due to limitations in studies with HIV-infected individuals, animal studies are needed. To date, the experimental infection of rhesus macaques (Macaca mulatta) with simian immunodefiency virus (SIV) is the best animal model for HIV/AIDS research. The aim of this work was to analyse frequencies, phenotype and the possible function of granzyme B-expressing B cells in healthy and SIV-infected rhesus macaques. Then B cells were purified using magnetic cell separation and different stimulation protocols were applied to induce granzyme B expression in vitro. Finally Co-culture experiments of these in vitro induced granzyme B-expressing B cells with T cells performed. Furthermore, we aimed at analyzing these cells in so-called long-term survivors (LTS), which lack disease progression in the absence of antiretroviral therapy, but can suddenly lose this status and progress to AIDS. By using multicolor flow cytometry the phenotype and frequencies of granzyme B-expressing B cells have been assessed and correlated with other immunologic parameters. Similar to HIV patients, significantly higher frequencies of these cells have been found in the blood of chronically SIV-infected rhesus monkeys compared with uninfected healthy ones. These frequencies correlated with plasma viral load and inversely with absolute CD4 T-cell counts. When investigating GrB^+ B cells in different compartments, levels were highest in blood, spleen and bone marrow, but considerably lower in lymph nodes and tonsils. Analysis of expression of various surface markers on this particular B-cell subset in SIV-infected macaques revealed differences between the phenotype in macaques and in humans. GrB^+ B cells in SIV-infected rhesus macaques exhibit an elevated expression of CD5, CD10, CD25 and CD27, while expression of CD19, CD185 and HLA-DR is reduced. In contrast to human GrB^+ B cells, a significantly increased expression of CD43 and CD86 has not been observed. B-cell receptor stimulation in combination with IL-21 of purified B cells from healthy animals led to the induction of GrB expression. Furthermore, initial functional analyses indicated a regulatory role on T-cell proliferation. Overall, this data pave the way for longitudinal analyses including studies on the functionality of GrB^+ B cells in the nonhuman primate model for AIDS.

Keywords: SIV, Rhesus Macaques, Long Term-Survivors, GrB^+ B cells, Flow Cytometry.

To my Family
and to
Philip Hagmann…

Who entered my Life and let me see everything from a better, more beautiful perspective.

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Introduction

HIV/AIDS is considered to be one of the most devastating infectious diseases that have emerged in recent history **(Barré-Sinoussi, 1983)**. Approximately, 36.9 million people are living with HIV, including 2 million people, who had been recently infected, and 1.2 million people having died from AIDS-related causes in 2014 **(WHO)**.

HIV infection results in a significant dysregulation of $CD4^+$ and $CD8^+$ T cells, dendritic cells and B cells **(Pantaleo, 1996)**. Dysregulation of B cells leads to high numbers of polyclonal activated B cells with hyperreactivity and hypergammaglobulinemia **(Moir, 2009)**. These hypereactive B cells are characterized by impairment in neoantigen and recall antigen B cell responsiveness **(Moir, 2009; Shirai, 1992)**.

Recently, a rare subset of B cells, called B regulatory cells (Bregs), was identified in mice and humans **(Blair; Mizoguchi, 2002)**. These Bregs were demonstrated to be Interleukin (IL-10) and granzyme B (GrB) producers**(Vadasz, 2014)**, although the latter commonly represents a major key component of NK (natural killer) cells and CTLs (cytotoxic T lymphocytes) **(M. Hagn, and Jahrsdörfer,B., 2012)**.

So far, the immunological function of these granzyme B-expressing (GrB^+) B cells remains elusive and may range from antiviral or cytotoxic and autoregulatory to regulatory functions**(Kaltenmeier, 2015; Vadasz, 2014)**. Recent studies discovered a large number of circulating GrB^+ B cells in the peripheral blood of HIV patients, although they are negligible in healthy people **(Kaltenmeier, 2015)**.

As studies with HIV-infected individuals are limited regarding routine sampling or collecting samples other than blood, animal studies are needed. So far, the most widely used animal model for HIV research is the experimental infection of rhesus macaques (*Macaca mulatta*) with Simian immunodeficiency virus (SIV).

This thesis shows for the first time the existence of GrB^+ B cells in rhesus macaques and aimed at analyzing these cells in both naïve and SIV-infected rhesus macaques. Using multicolor flow cytometry frequencies of GrB^+ B cell in the periphery as well as primary and secondary lymphoid organs were assessed and a comprehensive phenotypic characterization was performed. Furthermore, possible correlations with markers prognostic for disease progression and studies regarding their functionality were performed.

Review of Literature

1. HIV and AIDS

In 1981, the United States of America announced more frequent reports about a newly acquired immune deficiency syndrome (AIDS) among homosexual men and drug addicts, which is clinically manifested by mucosal candidiasis, Pneumocystis carinii-pneumonia (PCP) (current nomenclature: Pneumocystis jiroveci), multiple viral infections and the development of Kaposi's sarcoma **(Gottlieb, 1981; Masur, 1981; Siegal, 1981)**. During this epidemic, USA health authorities suggested that AIDS is accompanied with lowering the lymphocyte count, especially $CD4^+$ T helper cells.

In the following years, weekly reports of the Center for Disease Control and Prevention (CDC) revealed new cases of AIDS **(CDC, 1981)** until they discovered the causative virus for this disease in 1983. In 1986 this T-lymphotropic virus was finally designed as Human Immunodeficiency Virus (HIV) **(Coffin, 1986)**.

In 1986 a new HIV was isolated from a patient from Senegal **(Clavel, 1986)** and named HIV-2. HIV was then renamed analogously HIV-1.

So far, the origin of HIV-1 and HIV-2 remains elusive. The most common and main theory is different simian immunodefiency virus (SIV) strains represent precursors for both types of HIV, which have been transferred from monkeys to humans as a zoonotic disease, and have been adapted to suit the human host. It is unclear how exactly these events have taken place. Causes might have been the consumption of monkey meat especially in Africa.

According to the most recent reports in 2015, 37 million are now living with HIV **(WHO/UNAIDS)**. Sub-Saharan Africa remains the most affected region, with nearly 1 in every 20 adult living with HIV and accounting for nearly 71% of the people living with HIV worldwide **(WHO 2013)** (Fig. 1).

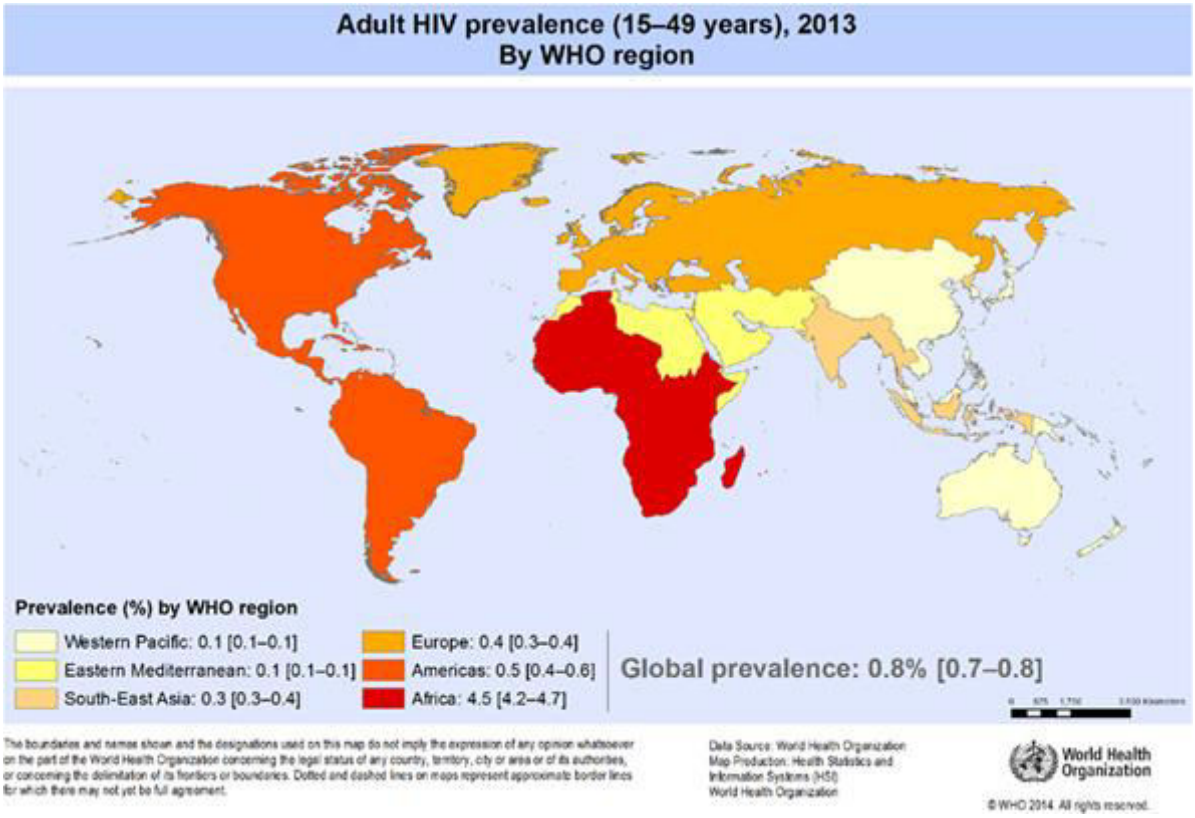

Figure 1: Worldwide distribution of HIV infections. According to UNAIDS and WHO AIDS epidemic update 2013.

Untreated HIV infections undergo a classical three-phase course of infection (Fig. 2) **(Pantaleo, 1996)**. In most cases, the acute phase of the disease starts few days to few weeks after infection with flu-like symptoms such as fever, malaise, lymphadenopathy, weight loss and myalgia and lasts up to four weeks **(Cooper, 1985)**. This phase is mainly characterized by a general immune activation accompanied with high viral load reaching peak viremia. In addition, there is a significant massive loss of $CD4^+$ T cells, especially in the intestinal mucosa, which was first described in the SIV model in rhesus macaques (*Macaca mulatta*, RM) **(Kewenig, 1999; Veazey RS, 1998)** and later was confirmed in HIV patients **(Brenchley, 2004; Guadalupe, 2003; Lim, 1993)**.