T cell dynamics and HIV specific CLT responses in Ethiopians
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Chapter 1

General Introduction

1.1. T cell immunity

T lymphocytes play a critical role in the regulation of immune responses and are responsible for mediating many of the effector mechanisms of the immune system. T cells follow a maturation process in the thymus, which involves a series of processes including T cell receptor (TCR) gene rearrangements, resulting in TCR of various specificities to specifically recognize the multitude of pathogens in the environment. Once they have completed their development in the thymus, naïve T cells enter the blood stream and recirculate between blood and peripheral lymphoid tissues until they encounter antigen.

The two major subsets of T cells comprising the cellular arm of the immune response, CD4+ and CD8+ T cells, recognize antigens in the form of peptide fragments that are presented at the cell surface bound to Major Histocompatibility complex (MHC) class II and MHC class I molecules, respectively. When the antigen-specific T cell receptor on the T cell surface interacts with the appropriate peptide-MHC complex, it triggers a cascade of intracellular signalling pathways. T cell activation also requires a second co-stimulatory signal such as the interaction between the surface markers CD28 on the T cell and CD80 on the antigen presenting cell (reviewed in ref 1). Activation of T cells can lead to cellular proliferation, lymphokine secretion by the T cell and expression of antigens associated with the activated state. IL-2 produced by the activated cells themselves drives proliferation of activated cells. Alternatively, in the case of cytotoxic T lymphocytes (CTL), interaction with antigen via the specific TCR leads to destruction of target cells. CD8+ T cells, when they recognize pathogen-derived small peptides (~9 amino acids) bound to MHC I, perform their effector function by releasing effector molecules such as the cytolysins perforin and granzymes, antiviral cytokines such as IFN-γ and TNF-α which inhibit viral replication, and the chemokines RANTES, MIP-1α and MIP-1β, which inhibit viral entry in a competitive manner (2, 3). They also express the TNF family membrane bound effector molecule, Fas ligand (CD95L), to induce apoptosis of Fas (CD95) expressing target cells. Effector functions of CD4+ T cells are more complex. CD4+ T cell activation leads to differentiation of a proliferating CD4+ T cell into a TH1 cell which activates macrophages and mediate cell-mediated immunity or TH2 cells, which activate B cells and mediate humoral immunity.

Activation of T cells with a specific antigen not only leads to proliferation and differentiation of naïve T cells into effector cells that eliminate the pathogen, it also leads to the generation of memory T cells which provide a more rapid and effective response up on antigenic re-challenge. The expression of isoforms of the leukocyte common antigen CD45 and a T cell activation antigen CD27 has been utilized as phenotypic criterion to discriminate distinct stages of postthymic human T cell differentiation (4). Naïve T Cells can be phenotypically characterized by the co-expression of CD45RA, the high molecular weight isoform of CD45, and CD27. Upon activation with their specific antigen, the naïve T-cells start to divide and differentiate into effector cells (CD45RA+CD27-) or convert into memory cells by losing CD45RA and expressing CD45RO antigen (CD45RA-CD27+). Further down regulation of CD27 antigen is believed to result from repeated antigenic challenge and is a characteristic feature of T cells with both memory and effector function (4-6).

In addition, due to their differential migration pattern in the body, naïve, memory and effector T cells differentially express adhesion and homing molecules. Naïve T cells need to travel through
the lymphoid tissues to encounter antigen and antigen presenting cells and hence are characterized by the expression of CD62L, which mediate homing to peripheral lymphoid tissues. On the other hand, memory and effector T cells migrate to all tissues in the body, particularly epithelial surfaces where pathogens are likely to be re-encountered.

1.2. Immunologic characterization of healthy Ethiopians
Following the first observation of CD4 T-lymphocytopenia without opportunistic infections in HIV seronegative Ethiopian immigrants to Israel (7), and a case report of idiopathic CD4+ T-lymphocytopenia in an HIV-negative Ethiopian with AIDS-like symptoms in Italy (8), several studies have been carried out to characterize the immunologic status of Ethiopians. These include comparative studies with Israelis (9) and Swedish (10), which demonstrated a perturbed immune system in Ethiopians and a series of studies have been carried out on Ethiopian Jews immigrated to Israel (9, 11-13). Although not truly representative of the multi-ethnic nation of Ethiopia, studies on Ethiopian Jews give a clue as to the general immune status of Ethiopians. The findings include: low CD4+ and high CD8+ T cell counts, low naive CD4+ T cell counts, increased CD45RO+ (memory) CD4+ T cell counts, decreased proportions of the co-stimulatory molecule CD28+ on CD8+ cells, increased expression of activation markers (HLA-DR and CD38), eosinophilia, increased levels of serum immunoglobulin E (IgE), increased IL-4 and IL-10 and soluble tumor necrosis factor (TNF) receptors with decreased secretion of IFN-γ, suggesting a persistently activated state of the immune system and polarization of the immune response towards a dominant Th2 profile which promotes humoral responses (9, 11, 12). Bentwich et al attributed these immune dysregulations to endemic chronic infections, especially helminthic infections (9, 11, 12). The activated immune status caused by chronic helminth infections has been shown to impair signal transduction and to cause T cell anergy, which could gradually be restored following anti-helminthic treatment (13). Deworming of Ethiopians living in Ethiopia has indeed been shown to lead to improvement of in vitro T cell responses (14) and to reduced expression of activation markers (HLA-DR and CD38) (15) after deworming have been demonstrated, lending support to the hypothesis of Bentwich and co-workers (9, 11, 12). In addition, a significant decrease in plasma HIV load after successful deworming has been observed in HIV infected Ethiopians (16).

The setting of an HIV/AIDS cohort study by the Ethio-Netherlands AIDS Research Project (ENARP), which recruited more than 1600 factory workers at two sites in Ethiopia, created an excellent opportunity for a more detailed investigation and to make comparisons with healthy Dutch controls (15, 17-22). These studies confirmed most of the observations on Ethiopian Jews such as the low CD4 T cell counts and signs of persistent activation of the immune system in Ethiopians. Additionally, CD4+ T cell counts were lower in HIV-positive Ethiopians compared to HIV infected Caucasians after seroconversion and at stage 1 and 2 of the WHO staging system for HIV infection and disease (17, 23).

1.3. HIV/AIDS situation
More than 20 years after the first clinical evidence of AIDS, combating HIV/AIDS is still a major global challenge. Worldwide, 34-46 million people are estimated to be living with HIV/AIDS, of which 60-75 % are from sub-Saharan African origin (24). Ethiopia, like most sub Saharan African countries, has been experiencing a severe HIV/AIDS epidemic starting from the mid 1980's. Unlike the neighbouring East African countries, the HIV epidemic in Ethiopia is predominantly of subtype C (25-28) with a genetic variant called C' (23, 29). The estimated time of introduction of HIV-1 to the country, 1982/83, (30, 31), coincides with the finding of the first
HIV-1 antibody positive sera which dated back to 1984 (32). The epidemic has spread rapidly and 2.2 million Ethiopians, including 200,000 children, are estimated to live with the virus (33). The primary modes of transmission of HIV in Ethiopia are heterosexual contacts and prenatal/mother-to-child transmission (33). Although the actual magnitude of the problem has yet to be assessed, harmful indigenous practices and unsafe needle injection may be considered to be mechanisms for the spread of the virus in view of their widespread practice in Ethiopia (34).

1.4. HIV specific T cell responses
Several direct and indirect evidences point to a key role by cytotoxic T lymphocytes (CTL) and T helper responses in the containment and suppression of viremia, both in acute and chronic phases of HIV infection. High CTL responses have been observed in highly exposed persistently seronegative African sex workers (35, 36), injecting drug users (37) and infants born to HIV infected mothers (38). The maintenance of seronegativity despite exposure to HIV has also been observed in health care workers occupationally exposed to HIV-contaminated body fluids (39, 40) and sexual partners of HIV infected individuals (41, 42). Moreover maintenance of strong HIV specific T cell responses has been associated with long term survival (43, 44). Most direct evidences came from animal models in which depletion of CD8+ T cells coincided with high viremia, which was again suppressed coincident with the reappearance of antigen specific CD8+ T cells (45-47). As a result, HIV vaccine strategies have focussed on the induction of sustainable T cell responses (48-50) and incorporation of dominant CTL epitope sequences into candidate vaccines is, therefore, vital.

Since both the host genetic background and the types of circulating HIV clades in a given population affect HIV specific T cell immunity, prior knowledge on the HLA frequencies in the population and genetic characterization of the circulating HIV clade are of importance. In addition, although cross-clade protection has been reported (36, 48, 51, 52) the types of dominant and subdominant CTL epitopes vary between populations (53-55), because of variances in the distribution of HLA alleles. In this regard, the circulating clade in Ethiopia is well characterized (23, 25-29); however, data on HLA types and mapping of immunodominant epitopes were lacking so far.

1.5. Scope of the thesis
This thesis consists of two parts. The first part (part I) deals with questions related to the general immune status of healthy Ethiopians, as this information is useful to understand immune alterations in HIV infection. In the second part, since there is an urgent need for the design of appropriate vaccines especially in the poorest parts of the world where the impact of HIV is highly devastating, we determined the frequencies of HLA types of Ethiopians and screened HIV infected Ethiopians to identify HIV Gag peptides that give dominant and sub-dominant T cell responses. More specifically the following questions were addressed in this thesis:

To address if the issue of low CD4 counts and persistently activated immune system reported on adult Ethiopians is a general phenomenon in the country, we determined distribution of lymphocyte subpopulations in two geographically distinct settings, as described in chapter II. In addition, to determine if there is any genetic basis for the immune dysregulations observed in healthy adult Ethiopians, we studied the distribution of T cell differentiation/maturation markers and T cell activation status in Ethiopians from birth (cord blood) to adulthood, as detailed in chapter III. Furthermore, we performed an in depth study by measuring multiple markers of chronic immune activation and markers to quantify T cells that are recently migrated from the
thymus at various age groups ranging from birth up to adulthood and compared these data with those obtained from age matched healthy Dutch (chapter IV).

As background knowledge to assess dominant and subdominant HIV proteins that can elicit protective immune response in HIV infected Ethiopians, we determined the frequencies of HLA types in HIV infected and non-infected Ethiopians (Chapter V). In addition, to identify CTL epitopes commonly targeted by subtype C infected Ethiopians, we screened PBMCs from 50 HIV infected Ethiopians for HIV-1 Gag specific T cell responses using the Elispot assay (Chapter VI). Furthermore, to evaluate the role of HIV specific T cell responses in HIV disease progression in subtype C infected Ethiopians, we performed a longitudinal analysis of HIV specific responses in a subgroup of subjects with relatively fast and slow CD4 decline as presented in chapter VII.

Finally, chapter VIII gives a general discussion of the various studies included in this thesis with a major emphasis on vaccination strategies to improve the antiviral immune response and future areas of research are indicated.

References


