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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. Immunolologic 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 naïve 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 immunoglubulin 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). Bentwitch 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 Bentwitch 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

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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 indepth 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

1. Hickling JK. Measuring human T-lymphocyte function. Exp Rev Mol Med. 1998; 13 Oct, www.ermm.cbcu.cam.ac.uk/jhc/txt001jhc.htm
2. Yang OO, Walker BD. CD8+ cells in HIV pathogenesis: cytolytic and non-cytolytic inhibition of viral replication. Adv Immunol. 1997;66:273-311.
3. Cocchi F, DeVico AL, Garzino-Demo A, et al. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. Science. 1995;270:1811-1815.
4. Hamann D, Baars PA, Rep MHG, Hooibrink B, Kerkhof-Garde SR, Klein MR, and Van Lier RAW. Phenotypic and functional separation of memory and effector human CD8+ T cells. J Exp Med 1997; 186:1-12.
5. Baars PA, Maurice MM, Rep M, Hooibrink B, and Van Lier RAW. Heterogeneity of the circulating human CD4+ T cell population: further evidence that the CD4+CD45Ra-CD27- T cell subset contains specialized primed T cells. J Immunol 1995; 154:17-25.
6. Hamann D, Roos MT, van Lier RAW. Faces and phases of human peripheral T-cell differentiation. Immunol Today 1999; 20:177-180.
7. Pollack S, Fuad B, Etzioni A. CD4 T-lymphocytopenia without opportunistic infections in HIV-seronegative Ethiopian immigrants to Israel. Lancet. 1993; 342:50.
8. Montella F, Viola P, Recchia O, Di Sora F, Rezza G. CD4+ T-lymphocytopenia and severe infections in an HIV-negative Ethiopian man. AIDS 1994; 8:390-91.
9. Kalinkovich A, Weisman Z, Greenberg Z, et al. Decreased CD4 and increased CD8 counts with T cell activation is associated with chronic helminth infection. Clin Exp Immunol. 1998; 114:414-421.
10. Worku S, Christensson B, Bjorkman A, Islam D. Higher proportions of CD8+ T cells in the blood in healthy adults from Ethiopia and Bangladesh compared with Sweden. Trans Roy Soc Trop Med Hyg. 1997; 91:618-622.
11. Bentwich A, Weisman Z, Moroz C, Bar-Yehuda S, Kalinkovich A. Immune dysregulation in Ethiopian immigrants in Israel: relevance to helminthic infections? Clin Exp Immunol. 1996; 103:239-243.
12. Bentwich Z, Weisman Z, Grossman A, Galai N. and Kalinkovich A. Pathogenesis of AIDS in Africa-Lessons from the Ethiopian immigrants in Israel. The Immunologist 1997; 5:21-26.

13. Brokow G, Leng Q, Weisman Z, et al. Chronic immune activation associated with intestinal helminth infections results in impaired signal transduction and anergy. J Clin Invest. 2000; 106:1053-1060.
14. Elias D, Wolday D, Akuffo H, Petros B, Bronner U, Britton S. Effect of deworming on human T cell responses to mycobacterial antigens in helminth exposed individuals before and after Bacille Calmette-Guerin (BCG) vaccination. Clin Exp Immunol. 2001; 123:219-225.
15. Kassu A, Tsegaye A, Wolday D, et al. Role of incidental and/or cured intestinal parasitic infections on profile of CD4+ and CD8+ T-cell subsets and activation status in HIV-1 infected and uninfected adult Ethiopians. Clin Exp Immunol. 2003; 132:113-119.
16. Wolday D, Mayaan S, G/Mariam Z, et al. Treatment of intestinal worms is associated with decreased HIV plasma viral load. J Acquir Immune Defic Syndr. 2002; 31:56-62.
17. Kassa E, Rinke de Wit TF, Hailu E, et al. Evaluation of the World Health Organization staging system for HIV infection and disease in Ethiopia: association between clinical stages and laboratory markers. AIDS 1999; 13:381-389.
18. Tsegaye A, Messele T, Tilahun T, et al. Immunohematological reference ranges for adult Ethiopians. Clin Diag Lab Immunol. 1999; 6:410-414.
19. Messele T, Abdulkadir M, Fontanet AL, et al. Reduced naïve and increased activated CD4 and CD8 cells in healthy adult Ethiopians compared with their Dutch counterparts. Clin Exp Immunol. 1999;115:443-450.
20. Hazenberg MD, Otto SA, James WT, et al. Increased Cell Division But Not Thymic Dysfunction Rapidly Affects the TREC Content of the Naive T Cell Population in HIV-1 Infection. Nature Med. 2000; 6:1036-1042.
21. Kassu A, Tsegaye A, Petros B, et al. Distribution of lymphocyte subsets in healthy human immunodeficiency virus negative adult Ethiopians from two geographical locales. Clin Diagn Lab Immunol. 2001;8:1171-1176.
22. Tsegaye A, Wolday D, Otto S, et al. Immunophenotyping of blood lymphocytes at birth, during childhood and adulthood in HIV-1 uninfected Ethiopians. Clin Immonol. 2003;109:338-346.
23. Rinke de Wit, Tsegaye A, Wolday D, et al. Primary HIV-1 subtype C infection in Ethiopia. J Acquir Immune Deffic Syndr. 2002; 30:463-470.
24. UNAIDS. Report on the global HIV/AIDS epidemic. December 2003. UNAIDS, Geneva, Switzerland. 2003.
25. Ayehunie S, Johansson B, Salminen M, et al. HIV-1 in Ethiopia: phylogenetic divergence from other HIV-1 strains. Virus Genes 1991; 5:359-366.
26. Sherefa K, Sällberg M, and Sönnerborg A.. Evidence of no change in V3 loop antibody recognition pattern in HIV type-1-infected Ethiopians between 1988 and 1993. AIDS Res Hum Retroviruses 1994; 10:1551-1556.
27. Abebe A, Kuiken CL, Goudsmit J, et al. HIV type 1 subtype C in Addis Ababa, Ethiopia. AIDS Res Hum Retroviruses 1997; 13:1071-1075.
28. Hussein M, Abebe A, Pollakis G, Brouwer M, Petros B, Fontanet AL, Rinke de Wit TF. HIV-1 subtype C in commercial sex workers in Addis Ababa, Ethiopia. J Acquir Immune Deffic Syndr 2000; 23:120-127
29. Abebe A, Pollakis G, Fontanet AL, et al. Identification of a genetic subcluster of HIV type 1 subtype C (C') widespread in Ethiopia. AIDS Res Hum Retroviruses 2000; 16:1909-1914.
30. Abebe A, Lukashov VV, Pollakis G , et al Timing of the HIV-1 subtype C epidemic in Ethiopia based on early virus strains and subsequent virus diversification. AIDS 2001; 15:1555-1561.
31. Abebe A, Lukashov VV, Rinke de Wit TF, et al. Timing of Introduction into Ethiopia of Subcluster C' of HIV-1 Type 1 Subtype C. AIDS Res Hum Retroviruses 2001; 17:657-661.

32. Tsega E, Mengesha B, Nordfeldt E, Hansson BG, Lindberg J. Serological survey of HIV in Ethiopia. Ethiop Med J 1988; 26:179-84.

33. Ministry of Health (MOH). AIDS in Ethiopia, 2nd ed. p. 21-24, Disease Prevention and Control Department, MOH, Addis Ababa, Ethiopia, 2002.

34. OkubEgzi G. and Singh S. Establishing an HIV/AIDS programme in developing countries:the Ethiopian experience. AIDS 2002;16:1575-1586.

35. Rowland-Jones SL, Sutton J, Ariyoshi K, et al. HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women. Nat Med 1995; 1:59-64.

36. Rowland-Jones SL, Dong T, Fowke KR, et al. Cytotoxic T cell responses to multiple conserved HIV epitopes in HIV-resistant prostitutes in Nairobi. J Clin Invest 1998; 102:1758-1765.

37. Makedonas G, Bruneau J, Lin H, Sekaly R-P, Lamothe F, and Bernard NF. HIV-specific CD8 T-cell activity in uninfected injection drug users is associated with maintenance of seronegativity. AIDS 2002; 16:1595-1602.

38. Rowland-Jones SL, Nixon DF, Aldhous MC. HIV-specific cytotoxic T-cell activity in an HIV-exposed but uninfected infant. Lancet 1993; 341:860-861.

39. Clerici M, Levin JM, Kessler HA, et al. HIV-specific T helper activity in seronegative health care workers exposed to contaminated blood. JAMA 1994; 271:42-46.

40. Pinto L, Sullivan J, Berzofsky JA, et al. Env-specific cytotoxic T lymphocyte responses in HIV seronegative healthcare workers occupationally exposed to HIV-contaminated body fluids. J Clin Invest 1995; 96:867-876.

41. Bernard NF, Yannakis CM, Lee JS, Tsoukas CM. HIV-specific cytotoxic T lymphocyte activity in HIV exposed seronegative persons. J Infect Dis 1999; 179:538-547.

42. Goh WC, Markee J, Akridge RE, et al. Protection against human immunodeficience virus type 1 infection in persons with repeated exposure: evidence for T cell immunity in the absence of inherited CCR5 coreceptor defects. J Infect Dis 1999; 179:548-557.

43. Klein MR, van Baalen CA, Holwerda AM, et al. Kinetics of Gag-specific cytotoxic T lymphocyte responses during the clinical course of HIV-1 infection: a longitudinal analysis of rapid progressors and long-term asymptomatics. J Exp Med 1995;181:1365-1372.

44. Gea-Banacloche JC, Migueles SA, Martino L, et al. Maintenance of large numbers of virus-specific CD8+ T cells in HIV-infected progressors and long-term nonprogressors. J Immunol 2000;165:1082-1092.

45. Jin X, Bauer DE, Tuttleton SE, Lewin S, Gettie A, et al Dramatic rise in plasma viremia after CD8+ T cell depletion in simian immunodefficiency virus-infected macaques. J Exp Med 1999; 189:991-998.

46. Schmitz JE, Kuroda MJ, Santra S, et al. Control of viremia in simian immunodeficiency virus infection by CD8+ T lymphocytes. Science 1999; 283:857-860.

47. Madden LJ, Zandonatti MA, Flynn CT, et al. CD8+ T cell depletion amplifies the acute retroviral syndrome. J Neurovirol 2004; 10 (suppl):58-66.

48. Ferrari G, Kostyu DD, Cox J, Dawsom DV, Flores J, Weinhold KJ, and Osmanov S. Identification of highly conserved and broadly cross-reactive HIV type 1 Cytotoxic T Lymphocyte epitopes as candidate immunogens for inclusion in *Mycobacterium bovis* BCG-vectored HIV vaccines. AIDS Res Hum Retroviruses 2000; 16:1433-1443.

49. McMichael AJ and Hanke T. HIV vaccines 1983-2003. Nature Med 2003; 9:874-880.

50. Berzofsky JA, Ahlers JD, Janik J, et al. Progress on new vaccine strategies against chronic viral infections. J Clin Invest 2004; 114:450-461.

51. Cao H, mani I, Vvincent R, et al. Cellular immunity to Human Immunodeficiency Virus type 1 (HIV-1) clades: relevance to HIV-1 vaccine trials in Uganda. J Infec Dis 2000; 182:1350-56.

52. Currier JR, Dowling WE, Wassuna KM, et al. Detection of high frequencies of HIV-1 cross-subtype reactive CD8 T lymphocytes in the peripheral blood of HIV-1-infected Kenyans. AIDS 2003;17:2149-2157.
53. Goulder PJR, Brander C, Annamalai K, et al. Differential narrow focusing of immunodominant HIV Gag-specific CTL responses in infected African and Caucasoid adults and children. J Virol 2000; 74:5679-5690.
54. Novitsky V, Rybak N, McLane MF, et al. Identification of Human Immunodeficiency Virus Type 1 Subtype C Gag-, Tat-, and Nef-Specific Elispot-Based Cytotoxic T-Lymphocyte Responses for AIDS Vaccine Design. J Virol 2001; 75:9210-9228.
55. Masemola A, Mashishi T, Khoury G, et al. Hierarchical targeting of subtype C human immunodeficiency virus type 1 proteins by CD8+ T cells: correlation with viral load. J Virol 2004; 78:3233-3243.