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REGULATION AND ROLE OF IL-7 PRODUCTION IN HIV-1 INFECTION

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*In memory of my father
to my mother and my parents in law
and to Mai, Dinh Duy and Duc Anh*

ABSTRACT

The concentration of interleukin-7 (IL-7) in human serum is elevated in various clinical conditions associated with lymphopenia, including HIV-1 infection. IL-7 is an essential factor for T cell differentiation and survival, and it was suggested that high serum IL-7 concentration may represent a homeostatic response to T cell depletion, which may promote T cell regeneration.

In order to increase our understanding on the regulation of IL-7 production, we investigated specimens from HIV-1 infected patients during chronic infection and in long term non-progressors (LTNPs). Serum IL-7 levels correlated with T-cell depletion in HIV-1 infected individuals. In some patients, we observed that serum IL-7 decreased upon progression to AIDS, suggesting a role for IL-7 in T-cell maintenance in sporadic cases. Interestingly, IL-7 levels were significantly lower in stable LTNPs than in patients who lost the LTNP status in a 3-year follow-up ($P<0.001$), indicating that serum IL-7 concentration might be a valuable marker for maintenance of the LTNP status.

The number of CD8+CD28- T cells increases significantly during aging and during HIV-1 infection. These cells have a reduced expression of the IL-7 receptor alpha (IL-7R α), as compared to CD8+CD28+ T cells. As CD8+CD28- T cells have been associated with dendritic and T cell suppression, we analyzed whether an increase in CD8+CD28- T cell numbers during HIV-1 infection could lead to impaired T cell responses. Peripheral blood CD8+CD28- T cells of both HIV-infected and non-infected individuals promoted dendritic cell activation. The CD8+CD28- T cell accumulation during HIV-1 infection may thus contribute to inflammatory reactions and immune activation.

Stromal cells and intestinal epithelial cells are known to produce IL-7. The mechanisms and cellular factors regulating IL-7 production are still unclear. We assessed whether IL-1 β and IFN- γ , cytokines produced during inflammatory conditions, may impact on IL-7 production. We used human intestinal epithelial cells (DLD-1 cell line) and bone marrow stromal cells (HS27 cell line) to evaluate IL-7 production at the mRNA and protein levels. To assess whether treatment of HS27 cells with IL-1 β and/or IFN- γ leads to changes in the gene expression of cytokines, Toll-like receptors (TLRs) and chemokines, we analysed gene expression profiles using the whole-genome microarray Human Gene 1.0 ST. We found that IFN- γ enhanced the expression of IL-7 protein and mRNA ($P<0.001$) in both cell lines. IL-1 β treatment led to a significant down-regulation ($P<0.001$) of IL-7 mRNA expression in both cell lines. The gene profiles revealed dramatic changes in expression of cytokines and their receptors, of IFN regulatory factors (IRF-1 and 2) and of important chemo-attractants for T cells. The microarray results were verified by additional methods. Our results were discussed in the setting of inflammation and T-cell survival in the gut compartment during HIV-1 infection where stromal and epithelial cells may produce factors that contribute to impaired IL-7 homeostasis and homing of T cells.

It was previously reported that IL-7 might stimulate T cell activation and CD95 mediated T cell apoptosis. HIV-1 infection leads to B cell abnormalities including increased apoptosis via the CD95 death receptor pathway and loss of memory B cells. Here we present a novel mechanism that can lead to increased B cell apoptosis in the presence of high IL-7 concentration. T cells cultured with IL-7 induced high CD95 expression on resting B cells together with an increased sensitivity to CD95 mediated apoptosis. As the mediator molecule responsible for B cell priming to CD95 mediated apoptosis we identified the cytokine IFN- γ that T cells secreted in response to IL-7. In the serum of HIV-1 infected individuals IL-7 and IFN- γ levels were in correlation and the level of both cytokines correlated with CD95 expression on circulating B lymphocytes in non-viremic individuals. These results indicate a potential link between IL-7 and the increased B cell apoptosis observed in HIV-1 infected individuals.

In conclusion the results presented in this PhD thesis highlight mechanisms of regulation of IL-7 production dependent on the number of circulating T cells and on the exposure of IL-7 producing cells to high levels of inflammatory cytokines. We also present data on the role of IL-7 in regulating CD95 expression and CD95 mediated apoptosis on B cells through IFN- γ produced by T cells; the impact of this finding on the outcome of IL-7 therapy during HIV-1 infection will be verified by ongoing clinical studies.

LIST OF PUBLICATIONS AND MANUSCRIPT

- I. Fluor C, Rethi B, **Thang PH**, Vivar N, Mowafi F, Lopalco L, Foppa CU, Karlsson A, Tambussi G, Chiodi F. *Relationship between serum IL-7 concentrations and lymphopenia upon different levels of HIV immune control*. AIDS. 2007 May 11;21(8):1048-50.
- II. Vivar N, **Thang PH**, Atlas A, Chiodi F, Rethi B. *Potential role of CD8+CD28- T lymphocytes in immune activation during HIV-1 infection*. AIDS. 2008 May 31;22(9):1083-6
- III. **Thang PH**, Ruffin N, Brodin D, Rethi B, Cam PD, Hien NT, Lopalco L, Vivar N, Chiodi F. *The role of IL-1beta in reduced IL-7 production by stromal and epithelial cells: a model for impaired T-cell numbers in the gut during HIV-1 infection*. J Intern Med. 2010 Aug;268(2):181-93.
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LIST OF ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
APC	Antigen presenting cell
ART	Antiretroviral therapy
Bcl-2	B-cell lymphoma 2
BM	Bone marrow
CCR	C-C chemokine receptor
CFSE	Carboxyfluorescein succinimidyl ester
CD	Cluster of differentiation
cDNA	Complementary DNA
CTL	Cytotoxic T cell
CXCL12	Chemokine C-X-C motif ligand 12
CXCR	C-X-C chemokine receptor
DC	Dendritic cell
DLD-1	Human intestinal epithelial cell line DLD-1
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
FRC	Fibroblast reticular cells
GALT	Gut-associated lymphoid tissue
GI	Gastrointestinal
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
HS27	Human bone marrow stromal cell line HS27
HTLV	Human T lymphotropic virus
ICAM-1	Inter-cellular adhesion molecule 1
IFN	Interferon
IL	Interleukin
IL-7R	IL-7 receptor
IRF	IFN regulatory factor
JAK	Janus kinase
LPS	Lipopolysaccharides
LTNP	Long-term non-progressor
mRNA	Messenger RNA
NK	Natural killer cell
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PCs	Plasma cells
RNA	Ribonucleic acid
SIV	Simian immunodeficiency virus
STAT	Signal transducer and activator of transcription factor
T reg	T regulatory cells
TLR	Toll-like receptor
VCAM1	Vascular cell adhesion protein 1
WHO	World Health Organization

1 INTRODUCTION

1.1 HIV AND THE PATHOGENESIS OF HIV INFECTION

1.1.1 Human immunodeficiency virus - HIV

In 1981, a new disease syndrome manifested in some previously healthy homosexual men in the United States characterized by unusual infections and cancers such as *Pneumocystis carinii* pneumonia and Kaposi's sarcoma. A marked reduction in cluster of differentiation (CD)4+ T cells was the common immunological dysfunction which characterized this group of patients. This new syndrome was named acquired immune deficiency syndrome (AIDS) (1). Two years later, the causative agent of AIDS was identified first by a group of scientists at the Pasteur Institut in Paris (2) and then by groups in the United States (3, 4). The causative agent of this syndrome was a retrovirus initially named human T lymphotropic virus type III (HTLV-III) until the new name human immunodeficiency virus (HIV) was agreed on by a panel of experts in the field.

HIV is a member of the Lentivirus sub-family in the Retroviridae family. The family is named Retrovirus since the viral ribonucleic acid (RNA) genome is transcribed into deoxyribonucleic acid (DNA) within the host cell using the viral enzyme reverse transcriptase (RT). This viral DNA is then transported to the nucleus and integrates into the cellular chromosome. The virus genome can be silent in the host cell until it is transcribed and the viral replication initiated.

HIV quickly spreads around the world. According to a report published in 2010, Joint United Nations Programme on HIV/AIDS (UNAIDS) estimated that there were 33.3 million adults and children living with HIV/AIDS world-wide, 2.6 million people newly infected with HIV in 2009 and 2.1 million AIDS-related deaths among adults and children in 2009 (5).

There are two types of HIV named HIV-1 and HIV-2 and the distinction between the two types is based on virus genetic differences. HIV-2 was identified two years after the discovery of HIV-1 (6). The genetic difference between HIV-2 and HIV-1 is about 40%. HIV-1 can also be divided into different groups M (main), O (outlier), and N (new or non M or O) and the genetic difference of virus groups is at least 30% from one to another, with variations mainly in the envelope genes (7). Recently, a new HIV-1

group was identified, named P, that is closely related to gorilla simian immunodeficiency virus (SIVgor) (8). The HIV-1 M group is further divided into subtypes (or clades) A-D, F-H, J, K and the subtypes differ by 15-20% in the genes (7). The genomic recombination of different HIV-1 subtypes created different HIV-1 recombinant viruses called circulating recombinant forms (CRFs) (9). Up to date more than 40 CRFs have been identified (www.hiv.lanl.gov). Of note, the original subtype E and I have been identified as recombinant subtypes. Virus recombination can occur after co-infection or super-infection of the same cell by two or more different virus subtypes (10).

HIV-1 caused the majority of HIV infection and distribution world-wide. By contrast, HIV-2 was primarily isolated from patients in West Africa although this virus type was also detected in individuals in other parts of the world including Europe, the United States, South America, and India. However, most of the HIV-2 cases detected in Europe and in the United States were from African migrations (11).

HIV-2 infection has a less pathogenic nature as compared to HIV-1 infection. There are several features that could possibly explain for the HIV-2 properties. During HIV-2 infection, lower viral load is generally detected in blood and genital fluids than the corresponding values found in HIV-1 infected subjects (12-14); the envelope glycoprotein (gp) 105 has a reduced affinity for the cell surface receptors, as compared to HIV-1 gp120 (15); patients infected with HIV-2 display a reduced level of immune activation (16, 17), low level of T cell apoptosis (16, 18) and lower CD8⁺ T cell cytotoxic immune response as compared to patients infected with HIV-1 (19).

1.1.2 HIV transmission

There are three main routes of HIV transmission, sexual contact, blood and blood products, and mother-to-child transmission. The transmission through the blood route has been proven to be most effective (20-24). However, HIV transmission through unprotected sexual intercourse is widely propagated. HIV transmission from mother-to-child can take place in utero, at delivery and during breast feeding. Without antiretroviral treatment (ART) the transmission from mother to child is believed to occur in 25-40% of pregnancy and delivery cases. The rate of transmission can be significantly reduced to < 10% or even to <2% with ART treatment of the mothers and children by Zidovudine or/and Nevirapine (20-24).

During primary HIV-1 infection, the virus infects a large number of CD4⁺ T cells. The HIV-1 virions replicate and spread very efficiently and the CD4⁺ T cells decrease sharply. During the acute phase of the HIV-1 infection, patients can experience influenza-like symptoms including fever, myalgia and skin rash. However, in most of cases those symptoms are minor and can be confused with other diseases or not at all recognized by the patient (25-28).

HIV-1 infects the host cell through the binding of the viral envelope gp120 to a high affinity receptor present at the cell membrane, the CD4 receptor. The CD4 receptor is expressed on T cells, macrophages, monocytes and dendritic cells (DCs) (29, 30). The HIV-1 gp120 molecules interacts with the CD4 receptors on the target cell and mediates the virus entry by fusing together the viral and the cell membranes; the C-C chemokine receptor (CCR) CCR5 or C-X-C chemokine receptor (CXCR) CXCR4 molecules act as co-receptors during this process. The interaction of the complex composed of CD4 and gp120 with a specific co-receptor triggers further conformational changes in the envelope glycoprotein complex. That process leads to the exposure and insertion of the hydrophobic gp41, the fusion peptide of the virus, into the membrane of the target cell.

HIV-1 isolates can preferentially infect either macrophages or T cells and accordingly they are named as macrophage tropic (M-tropic) or T cell tropic (T-tropic) isolates. A macrophage-tropic HIV-1 virus uses CCR5 receptor as co-receptor to attach and infect the cell and is accordingly classified as a R5 virus. HIV-1 viruses with the property of infecting T cell lines are called X4 viruses and infect T cells using CXCR4 as co-receptor. The X4 viruses are more cytopathic than the R5 viruses (31). Individuals who lack CCR5 expression on immune cells due to mutation in the CCR5 ($\Delta 32$) gene are resistant to R5 virus infection but are still susceptible to infection with X4 viruses. Some other chemokine coreceptors (e.g., CCR3, CCR2b) are known to act as primary or secondary attachment sites for both HIV-1 and HIV-2 isolates, but are not commonly involved in infection (31).

Some studies have shown that R5 viruses are generally observed in the blood during acute or early infection. However, during advanced disease progression and AIDS, approximately 50% of patients carry cytopathic X4 viruses in blood (32, 33). R5 viruses infect preferentially CCR5⁺CD4⁺ T cells in the gastrointestinal (GI) tract and R5 viruses can easily infect macrophages and DCs (7).

1.1.3 Immune response in HIV-1 infection

HIV-1 infection targets cells of the immunological system and renders them dysfunctional. The immunopathological manifestations of HIV-1 infection are generally a gradual reduction of CD4⁺ T cells in the peripheral blood as well as in the lymphoid tissues and gut-associated lymphoid tissue (GALT); enhanced B cells proliferation and hypergammabulinemia – features which may reflect immune activation occurring during chronic infection. An overt stage of immune deficiency is often accompanied by opportunistic infections and malignancies like Kaposi's sarcoma. The mechanisms through which HIV-1 induced immune activation is established over time leading to destruction of the immune system are depicted in Fig. 1.

1.1.3.1 *T cells*

CD4⁺ T cells loss is the main pathogenic feature of HIV-1 infection. At the acute phase of HIV-1 infection, there is a dramatic depletion of CD4⁺ T cells which can be detected in peripheral blood; thereafter the number of CD4⁺ T cells rebounds to certain levels after the initial HIV-1 burst decreases to a set point in the host body. Without any treatment, the CD4⁺ T cells are gradually lost during the following years of HIV-1 infection.

In SIV infection in macaques within a few days from infection, the virus rapidly migrates to the GALT where it induces a massive depletion of memory CD4⁺ T cells in the intestinal lamina propria (34, 35). The same picture of CD4⁺ T cells depletion in the GALT as the one occurring in SIV infected macaques was observed in humans at the early stage of HIV-1 infection (36-38).

Depletion of CD4⁺ T cells happens not only in peripheral blood and GALT, but also at other mucosal sites and in the lymph nodes. Studies in SIV and HIV-1 infection also demonstrated that the CD4⁺ T cells depletion is less pronounced in peripheral blood and lymph nodes as compare to GALT (37, 39).

There are some potential factors involved in the loss of CD4⁺ T cells in HIV-1 infection. The direct cytopathic effects of HIV-1 on CD4⁺ T cells and progenitor cells lead to cell death (40). HIV-1 induced apoptosis of the target cells and it was shown that HIV-1 encoded proteins induced apoptosis of infected and uninfected cells (41-43). In many cases apoptosis results from direct virus infection whereas bystander effects of the virus infection causes immune activation (25, 44). Destruction of the lymphoid

tissue reduces the production of new cells (45, 46). CD4+ T cells loss is the primary reason for occurrence of the opportunistic infections and cancers associated with HIV-1 infection.

CD8+ T cells so called cytotoxic T cells (CTLs) have been found to be increased in percentage as well as in absolute number in HIV-1 infection (47) and CD8+ specific T cells are distributed in different anatomical compartments in both humans and macaques. CD8+ specific T cells appear to be involved in the control of HIV-1 replication (48) and *in vitro* CD8+ T cells can control HIV-1 infection by inhibiting HIV-1 replication (49). Recent evidence also showed that early HIV-1 specific CD8+ T cell responses contribute to reduce HIV-1 viremia in plasma during the acute phase of HIV-1 infection (50). CD8+ T cells are also involved in adaptive immune function by killing the virus infected cells. CD8+ T cells produce some soluble factors such as RANTES (Regulated upon Activation, Normal T-cell Expressed, and Secreted), macrophage inflammatory protein (MIP)-1 α and MIP-1 β (51), and additional important cytokines like interferon gamma (IFN)- γ and tumor necrosis factor alpha (TNF)- α (52). It is important to underline that CD8+ T cells activities can be detrimental if they lead to lysis of autologous uninfected CD4+ T cell and antigen presenting cells (APCs). It was shown that the expression of the programmed death 1 protein (PD-1) on HIV-1 specific CD8+ T cells can reduce their cytotoxic function (53).

1.1.3.2 B cells

Another important component of HIV-1 pathogenesis is the damage occurring to B lymphocytes, which can be measured by altered phenotype and composition of different B cells populations, increased apoptosis of these cells and an abnormal pattern of activation (54). Phenotypic and functional alterations on B lymphocytes are often observed in chronically infected patients, but alterations of B cells are already detected during the acute phase of HIV-1 infection (55). A decline of memory B cells has previously been reported to occur in both children and adults infected with HIV-1 (56, 57); these cells are responsible for mounting and maintaining an adequate serological response to antigens previously encountered in life through natural infection or vaccination. The decline in B cells carrying immunological memory correlated to loss of antibody titers to measles, tetanus, and pneumococcal antigens, a process which already began during primary HIV-1 infection. The consequences of loss of memory B

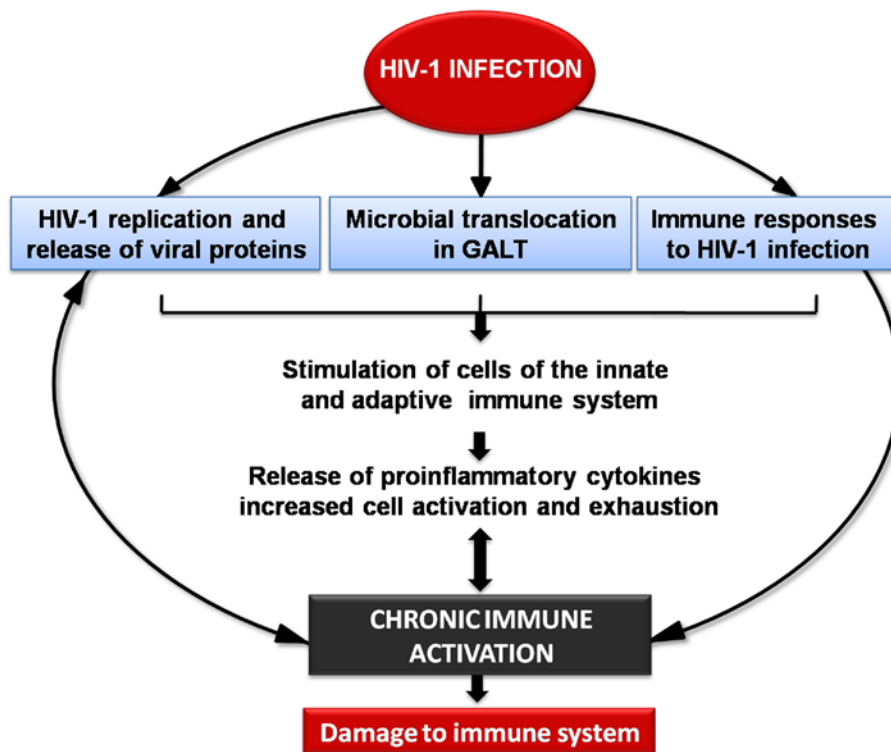


Figure 1. HIV-1 infection induces immune activation and destruction of the immune system. Due to HIV-1 infection, innate and adaptive immune cells become activated by different factors like viral proteins, microbial components translocated from the GALT and host immune responses. Activated cells release proinflammatory cytokines (IFN, TNF, IL-1, IL-6), which in turn, together with HIV-1 replication and host immune responses, contribute to chronic immune activation. Chronic immune activation increases HIV-1 replication and leads to immune cell exhaustion, finally causing damage to the immune system.

cells are not fully understood; the loss of memory B cells during HIV-1 infection may be mediated by up-regulation of CD95 (Fas) receptor and increased susceptibility to CD95 mediated apoptosis (55).

1.2 APOPTOSIS

Apoptosis, or programmed cell-death, provides a mechanism for removal of senescent cells without evoking inflammatory responses. Two main pathways have been identified for execution of apoptosis: 1) the intrinsic apoptotic pathway, also defined as the mitochondrial pathway is receptor-independent, and requires mitochondrial participation and 2) the extrinsic or death receptor dependent pathway, involves the interaction of a death receptor with its natural ligand (58). Apoptosis is regulated by

pro- and anti-apoptotic proteins. Caspase activation and cleavage of specific cellular substrates occur as result of both the intrinsic and extrinsic pathways, ultimately leading to chromatin condensation and DNA fragmentation. The process of apoptosis is completed when the apoptotic cells are removed by phagocytosis. Due to efficient phagocytosis, few apoptotic cells are found in healthy tissues despite a constant homeostatic turnover of senescent cells.

Different apoptotic pathways have been described to date, many of which overlap each other at the molecular level. The permeabilization of the mitochondrial outer membrane (MOMP) is a pivotal point of mitochondrial apoptosis pathway which triggers caspase activation resulting in irreversible events of cell apoptosis. This intrinsic apoptotic pathway involves the regulation of different important proteins of the B-cell lymphoma (Bcl)-2 family, which comprises, among others, the anti-apoptotic proteins Bcl-2, Bcl-xL and Bim and the pro-apoptotic proteins Bax and Bak (58). Extrinsic apoptosis can be initiated through activation of a number of membrane bound receptors; among them the CD95 protein which is expressed as a transmembrane receptor (59) and is ubiquitously expressed in the majority of cell types. The natural ligand for CD95 is CD95 ligand (CD95L) mainly expressed by activated T-cells, NK cells and macrophages. An important molecular component in the structure of death receptors is a cytoplasmic domain of approximately 80 residues named death domain which plays an important role for recruitment of molecules which initiate intracellular signalling leading to apoptosis.

CD95/CD95L interactions have been proposed to be necessary to down-regulate the number of reactive cells during contracting phase of immune responses (60). Aberrant apoptosis of T cells during HIV-1 infection leads to immunosuppression and susceptibility to opportunistic infections (61). Studies on the impact of viral infection on the GALT have demonstrated that apoptosis is a pivotal mechanism for HIV-1 driven destruction of mucosal CD4⁺ T cells. In addition, high levels of CD95/CD95L expression and CD95-mediated apoptosis were detected in lamina propria T cells (35).

1.3 INTERLEUKIN-7 AND IL-7 RECEPTOR

1.3.1 Interleukin-7

The Interleukin (IL)-7 belongs to the type 1 cytokines of the hematopoietic family. IL-7 is a non-redundant cytokine with important roles in lymphocytes development and survival and maintenance of peripheral lymphocytes homeostasis. IL-7 was first discovered in 1988 as a novel growth factor for precursor murine B cells produced in vitro from bone marrow derived stromal cells (62). Additional studies conducted later on showed that IL-7 has an effect on growth and differentiation of immature and mature T cells and fetal thymocyte clones (63, 64). IL-7 was demonstrated to be required for the survival and proliferation of mature and naive peripheral T cells (65).

Recently, an in vitro study was conducted by culturing peripheral blood mononuclear cell (PBMCs) in presence or absence of IL-7, followed by infection with an HIV-1 R5 strain (66). Interestingly, IL-7 increased the density of CXCR4 receptor at the CD4⁺ T cell surface and induced the switch of HIV-1 R5 virus to X4 virus.

Human IL-7 gene spans 6 exons located on the chromosome 8q12-13 encoding for a protein of 177 amino acids (aa) with molecular weight of 25 kilo Dalton (kD) in active form (67). Different IL-7 isoforms, defined as alternative splice variants, have been described; the different isoforms have been mapped in different human tissues suggesting an important role for these different isoforms in different disease conditions (68).

IL-7 is produced by non-lymphoid cells, and IL-7 messenger RNA (mRNA) has not been detected in lymphocytes during physiological conditions. In humans, different cell types are known to produce IL-7 and among them the IL-7 main source are stromal cells in the bone marrow (69, 70), intestinal epithelial cells (71, 72) and fibroblastic reticular cells (73). In addition, keratinocytes (74), peripheral blood DCs (75), follicular DCs, smooth muscle cells and endothelial cells (76) were also found to produce IL-7. Recently, a study showed that stimulation through Toll-like receptors (TLRs) of liver hepatocytes can induce production of IL-7 from hepatocytes (77). This mechanism of regulation via TLRs triggering appears to be unique for hepatocytes and the production of IL-7 by hepatocytes stimulated with lipopolysaccharides (LPS) appears to be transient. A study conducted ex-vivo has shown that IL-7 production by bone marrow stromal cells derived from HIV-1 infected patients during ART treatment was lower as compared to the levels detected

before ART (78). IL-7 was also shown to be produced by a small population of mice DCs regulating the niche size of CD4⁺ T cells in vivo (79).

How IL-7 is constitutively produced, and the mechanism for regulation of IL-7 production is yet not understood in detail. IFN- γ has been shown to up-regulate the IL-7 production in human intestinal epithelial cells through IFN regulatory factors 1 and 2 (IRF-1, 2) via an IFN regulatory factor element (IRF-E) acting on the 5' flanking region of the human IL-7 gene (80). Other factors like transforming grow factor- β (TGF- β) (81) and flagellin (82) have been reported to negatively modulate the IL-7 production. On the other hand, IL-1 and TNF- α have been shown to up-regulate IL-7 production from stromal cells (83). Recently, a study showed that in vivo, commensal microflora drives IFN- γ production by lymphocytes, which in turn promotes IL-7 production from intestinal epithelial cells and the survival of IL-7-dependent lymphocytes. Interestingly, the combination of IFN- γ with the commensal microflora promotes a steady-state IL-7 production in the intestine (84).

IL-7 is considered as an obligate survival factor for several subsets of progenitor and mature lymphoid cells. Mutations in the IL-7 gene or its receptor complex in mice resulted in impaired IL-7 production and IL-7 mediated signaling; upon these conditions the mice became severely lymphopenic, with T cell depletion at both primary and peripheral lymphoid sites and with T cell numbers decreased 10–20-folds (85). The absence of IL-7 triggered apoptotic processes in IL-7 dependent cells, as shown by increased annexin V binding to the cell membrane and DNA fragmentation (86). More recently it has been shown that IL-7 is required for homeostatic survival of peripheral T lymphocytes. Under T cell deficient conditions, naïve T cells were able to expand in mice lacking other cytokines, such as IL-4 or IL-15, but not in mice lacking IL-7, and the same was shown for memory CD8⁺ T cells (87).

1.3.2 IL-7 receptor

The IL-7 receptor (IL-7R) belongs to the cytokine receptor family. The IL-7R consists of two components: the IL-7 receptor alpha chain (α) also named CD127 and a common gamma chain (γ_c) (CD132) which is shared by the receptors for IL-2, IL-4, IL-7, IL9, IL-15 and IL-21. The cytoplasmic domains of the IL-7R α and γ_c are required for STAT5A/B activation which is followed by signal transduction. Studies conducted in mice have shown that IL-7R α and γ_c -deficient mice presented with similar features

characterized by diminished T cell numbers and impaired lymphocyte development (88). These data indicate that both IL-7R α and γ c are essential to mediate the biological effects of IL-7 on the target cells.

The IL-7R α chain is expressed on different cell types. In human hematopoietic cells, IL-7R α is expressed on developing T and B cells, mature T cells (both naïve and memory T cells), natural killer cell (NK) and DCs. Other human cell types such as intestinal epithelial cells, endothelial cells, bone marrow stromal cells, cancer cells of colorectal, lung, and breast origin also express the IL-7R α .

IL-7R α gene is located in human chromosome 5p13, which contains 8 exons; the IL-7R α gene encodes the IL-7R α proteins which is comprised of 439 aa and has a molecular weight of 49.5kD. The IL-7R α is a glycoprotein trans-membrane receptor including a single 25 aa transmembrane domain and a cytoplasmic tail of 195 aa.

IL-7R α expression on cell surface is regulated by different factors. It has been shown that the IL-7R α expression is increased by glucocorticoids in T cells and cell lines (89), by TNF in mice T cells, by IFN- α and IFN- β in mice and human cell lines (90). The expression of IL-7R α in T cells is suppressed by different inhibitors. The expression of the IL-7R α is suppressed by its ligand IL-7 (91) and also by some of the cytokines belonging to the common γ c receptors IL-2, IL-4, IL-15 and other pro-inflammatory and anti-inflammatory cytokines including IL-6 (91, 92). The T cell receptor (TCR) when activated also inhibits the IL-7R α expression (89, 93). The expression of IL-7R α on T cells is down-regulated during HIV-1 infection (94, 95). It has been shown that soluble HIV-1 Tat protein removes the IL-7R α from the surface of resting CD8⁺ T cells. HIV-1 Tat protein targets IL-7R α for degradation via the proteasome leading to reduced IL-7 signaling and impaired CD8⁺ T cell proliferation and function (95, 96). When IL-7R α is expressed in cells it binds to its specific ligand IL-7 and will induce cell survival and proliferation. However, over expression of IL-7R α is also associated with some negative effects such as induction of inflammatory bowel disease (97) and induction of lymphoma in transgenic mice (98). On the other hand, studies conducted in *gld* mice that lacked the IL-7R α expression, or where the IL-7R α expression was blocked, showed a pattern of inhibition of T cell proliferation and survival (99).

1.3.3 IL-7/IL-7R signaling pathways

When IL-7 binds to its specific receptor, different signaling pathways linked to the IL-7 receptor are activated in order to obtain different biological effects in the target cells (Fig. 2).

1.3.3.1 JAK-STAT pathways

- JAK3: Janus kinase (JAK) 3 is a protein belonging to the family of intracellular tyrosine kinases. JAK3, which is recognized as the first step in the signal transduction cascade from the IL-7 receptor, is constitutively associated with the carboxy-terminal region of the γ_c chain receptor. In humans, mutations in the JAK3 gene result in a disease similar to the XSCID (X-linked Severe Combined Immune Deficiency) caused by mutation in γ_c chain (100). The main function of the JAK3 signaling pathway is to protect the cell from apoptotic death.

- JAK1: JAK1 is associated with the IL-7R α chain and phosphorylated following IL-7 binding to the IL-7R α . The protein tyrosine kinase Pyk2, which is related to the focal adhesion kinase, was shown to be associated with JAK1 and to play a role in survival of a thymocyte cell line (101). JAK1 activity is required for the IL-7 mediated inhibition of TGF- β production and signaling by fibroblast (102). It has been shown that mice deficient in JAK1 exhibited severely impaired thymic development and no hematopoietic colony formation in response to IL-7 (103).

- STATs: STATs (Signal Transducer and Activator of Transcription factors), are a family of transcription factors containing SH2 domains that are involved in cytokine mediated signal transduction through the cytoplasmic region of cell surface receptors. There are 7 different members (STAT1 to STAT4, STAT5A, STAT5B and STAT6) in the STATs family which are activated through JAK.

It has been shown that IL-7 can activate STAT1 and STAT3 (104), but animals deficient in STAT1, 2, or 3 do not show defects in thymocyte development. The SH2 domain of STAT5 docks at tyrosine 449 of the IL-7R α to start the signaling pathway. STAT5 signaling pathway is known for its anti-apoptotic activity which is exerted through the regulation of expression of several Bcl-2 family members and caspases. It has been shown that IL-7 is required for the survival and development of T lymphocytes, IL-7 is also required for the survival of pre and pro B cells by inducing

the expression of the anti-apoptotic factors Bcl-2, Bcl-xL, Mcl-1 and by reducing the expression of the death proteins Bax, Bad and Bim (65).

1.3.3.2 PI3 kinase pathway

The phosphatidylinositol 3 (PI3) kinase is one of the downstream pathways of IL-7 that regulates cell survival and proliferation of different cell types. The PI3 kinase pathway has been demonstrated to be important for B and T cell development.

In human T cells, JAK3 associated with the p85 subunit of PI3 kinase following IL-7 stimulation leads to induction of PI3 kinase activation by phosphorylating p85. The activation of PI3 kinase is essential for IL-7 mediated survival and proliferation of human T cell precursors (65).

IL-7 induces activation of AKT, a key downstream target of PI3 kinase, in IL-7 dependent mouse thymocyte cell line (105) and human thymocytes (106). In turn, activated AKT phosphorylates the dead protein Bad, and p27. In addition, AKT also regulates an additional dead protein Bim, through the phosphorylation of forkhead transcription factor FoxO3. By regulating the expression of the dead proteins Bad, p27 and Bim, IL-7 is involved in the regulation of cell survival and cell proliferation through the PI3kinase/AKT pathway (65).

1.3.3.3 Src kinase pathway

The Src family protein kinases (SFKs) are membrane- associated non-receptor protein tyrosine kinases that include nine members Src, Lck, Hck, Fyn, Blk, Lyn, Fgr, Yes and Yrk. It has been shown that IL-7 activates Src kinases. IL-7 stimulation of pre- B cell lines leads to the activation of p59fyn and p53lyn (107). Binding of IL-7 to the IL-7R results in the activation of p56lck and p59fyn of Src kinases, but these are unlikely to be the only pathways responsible for the proliferation of activated T cells in response to IL-7 (108). In peripheral T cells, IL-7 provides signals in addition to the TCR signaling pathways mediated by lck/fyn for the cell survival and proliferation (109). However, the IL-7/IL-7R α activation of the Src kinase pathways leads to different degrees of cell proliferation and homeostasis (65).

1.3.3.4 IL-7 in metabolism

It has been shown that IL-7 participates in the maintenance of cellular metabolic activity through the cellular uptake of glucose. In a IL-7 dependent thymocyte line, glucose uptake was reduced following withdrawal of the cytokine (110). The expression of glucose transporter type 1 (GLUT1), a protein involved in cellular glucose transport, is regulated by the down-stream substrate of PI3 kinase, AKT. IL-7 promotes GLUT1 expression and increases glucose uptake in leukemic T cells through PI3K and ATK(65).

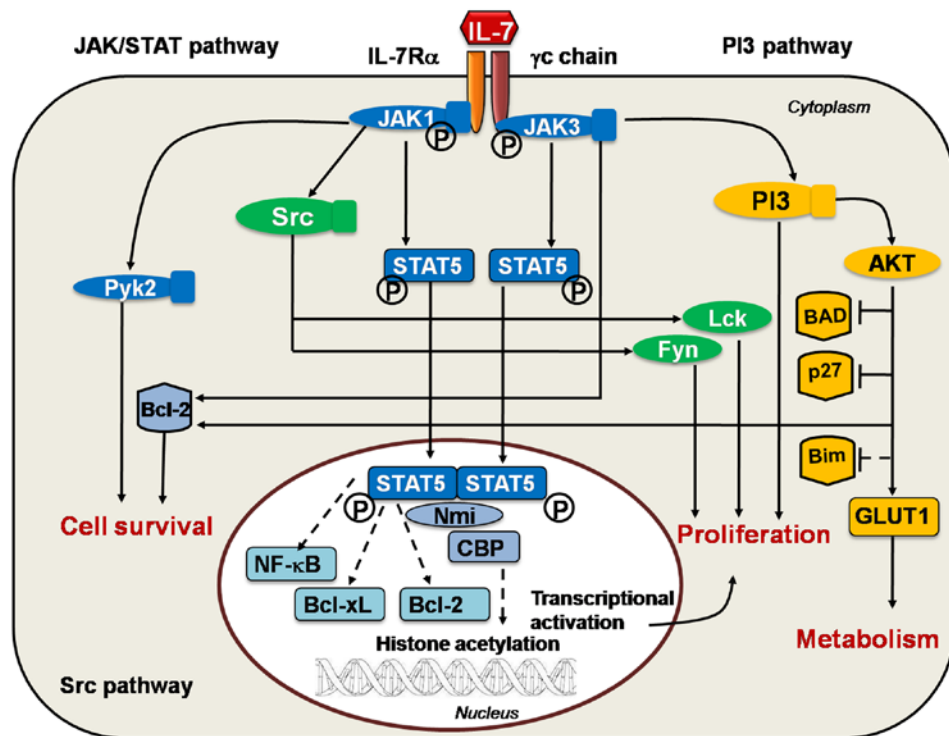


Figure 2. Outline of IL-7/IL-7R signaling pathways. Activation of different down-stream components of the IL-7R pathway can lead either to cell survival and/or proliferation.

1.3.4 IL-7 and IL-7Rα in lymphopenic condition

A study on the serum IL-7 levels in children, before and after 8 weeks from bone marrow transplantation, showed a strong inverse correlation between the circulating IL-7 levels and the absolute number of lymphocytes (111). This inverse correlation trend between the IL-7 levels and number of CD4⁺ T cells was also observed in children and adult patients receiving cancer therapy and HIV-1 infected patients (94, 112, 113). In

cancer patients treated with cytotoxic chemotherapy, the circulating IL-7 levels increased following CD4⁺ T cell depletion induced by chemotherapy; IL-7 serum levels returned to baseline following recovery of CD4⁺ T cell counts after completion of therapy.

In HIV-1 infected patients, elevated IL-7 level declines as recovery of the CD4⁺ T cell counts take place after effective ART. The inversed correlation of serum IL-7 levels with the numbers of CD8⁺ T cells and B cells is weaker as compared to CD4⁺ T cells (113). Similar relationships were not observed between the number of lymphocytes and other cytokines including IL-2, IL-4, IL-6, IL-2 and IL-15. This suggested that the relationship of IL-7 and lymphocyte number was unique, and reflected the role of IL-7 in regulation of T cell homeostasis, in stimulation of lymphocyte development and maintenance of peripheral T lymphocytes, especially CD4⁺ T cells.

As previously described, the binding of IL-7 to the IL-7R receptor leads to intracellular signaling. It has been shown that the IL-7R α is down-regulated in some chronic infections such as Epstein-Barr virus (EBV), cytomegalovirus (CMV), hepatitis C virus (HCV) and HIV-1 infection (114, 115). During lymphopenic condition associated with HIV-1 infection, there is a decrease in the IL-7R α expression on both CD4⁺ and CD8⁺ T cells (94, 116, 117). It has been shown that T cells expressing low levels of IL-7R α also express lower levels of the anti-apoptotic Bcl-2 molecule as compared with the IL-7R α -high T cells in the same donors (94).

The mechanisms leading to high level of circulating of IL-7 and IL-7R α down-regulation during HIV-1 infection are not yet clarified. There are however some possible explanations for these phenomena. An “altruistic” hypothesis has been proposed implying that a T cell that has been exposed to IL-7, and received a sufficient survival signal, would cease consuming IL-7 by down-regulating IL-7R expression thus allowing other cells to receive a survival signal (65). Moreover, since in HIV-1 infection the IL-7R α is down-regulated on T lymphocytes, the efficiency of IL-7 on T cells survival decreases due to lower consumption in spite of the high IL-7 levels in circulation (118). Another possible explanation is that during HIV-1 infection, IL-7 may be accumulated in view of the fact that the number of CD4⁺ T cells is reduced. The HIV-1 Tat protein acts by inducing IL-7R α down-regulation, even in presence of high IL-7 levels (119).

In HIV-1 infection the majority of IL-7R α low T cells are previously activated antigen-specific T cell clones in late differentiation stages. That suggests the chronic antigenic stimulation to provide a driving force for IL-7R α down regulation (119).

1.4 IMMUNE ACTIVATION IN THE GUT MUCOSA DURING HIV-1 INFECTION

The intestinal mucosa plays an important function as an immunological barrier to pathogens of the outside environment, and also permits a peaceful coexistence with the commensal flora. It has been shown that the GI tract is the largest lymphoid organ in the body, with an estimated surface area 200 times larger than that of the skin (120).

The epithelial layer of the GI tract consists of intestinal epithelial cells (IECs) connected by tight junctions, mucus-secreting goblet cells and antimicrobial-peptide-producing Paneth cells. Interspersed throughout the intestinal epithelium are the GALT, including Peyer's patches in the small intestine and isolated lymphoid follicles in the colon, which contain immunoglobulin (Ig) A-secreting plasma cells. These different cell populations create and support a mucus layer, containing IgA and antimicrobial peptides, which dramatically reduces the number of bacteria at the barrier between the epithelium and lumen (121).

HIV-1 infection causes damage on the GI tract structure. It has been shown that the HIV-1 Tat protein inhibited the glucose uptake of enterocytes and that the HIV-1 gp120 induced increase of calcium concentration in enterocytes leading to a decreased ability of intestinal epithelial cells to maintain the ionic balance. In chronic infection, HIV-1 causes the damage of intestinal epithelial barriers through apoptosis of enterocytes and decreased luminal defensin. Also in the gut there is a massive depletion of CD4⁺ T cells and a high number of infected CD4⁺ T cells which release virions continuously. Due to the damage of the epithelial barrier there is an increase in microbial translocation and an increased permeability of the intestinal epithelial barrier (122).

In acute infection: Studies conducted during the acute phase of SIV infection in rhesus macaques demonstrated a rapid and almost complete loss of CD4⁺ T cells from the intestinal lamina propria (39). The depletion of CD4⁺ T cells occurs at all mucosal surfaces examined regardless of the route of infection, and was found to be due to HIV-1 targeting of CCR5⁺ memory CD4⁺ T cells which are the largest proportion of mucosal

CD4⁺ T cells. It has been shown that 60% of the mucosal memory CD4⁺ T cells are infected at the peak of viremia during acute phase of SIV infections, and in the infected animals 80% of the infected cells are depleted within 4 days from the infection (123). During HIV-1 infection in human, it was shown that a substantial level of CD4⁺ T cell depletion occurs in the GI tract during HIV-1 infection; this pathogenic feature, which occurs preferentially within the CCR5⁺ T cells, can be found both during the early and the chronic phases of HIV-1 infection (37, 38). Patients treated with ART during the early stages of HIV-1 infection showed a more efficient reconstitution of CD4⁺ T cells in the GI tract than individuals treated with ART during chronic HIV-1 infection (124).

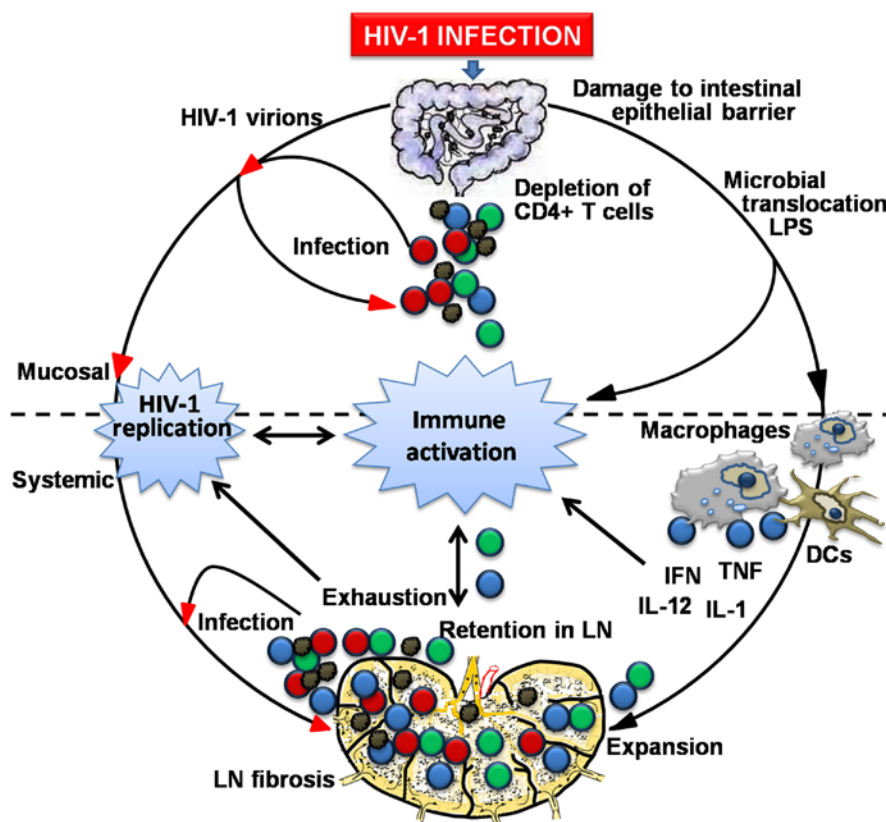


Figure 3. Immune responses in gut and HIV-1 pathogenesis. Acute HIV-1 infection leads to CD4⁺ T cell death (black) in the gut, which destructs the mucosal barrier and increases microbial translocation. Bacteria and bacterial components stimulate immune cells to produce pro-inflammatory cytokines, which contribute to chronic HIV-1 infection and immune activation. In turn, chronic immune activation leads to CD4⁺ (blue) and CD8⁺ (green) T cells expansion, thus creating more targets for direct infection of CD4⁺ T cells (red). Chronic immune activation stimulates HIV-1 replication and also results in lymph node fibrosis, which limits lymph node function to support healthy T cell homeostasis. Due to the fibrosis, CD4⁺ T cells are retained in lymph nodes and become targets for direct HIV-1 infection and die. DCs, dendritic cells; LPS, lipopolysaccharide; LN, lymph node; IL, interleukin; TNF, tumor necrosis factor; INF, interferon. (Adapted from Brenchley J, *Nature Immunology*, 2006).

Different mechanisms are proposed to explain the loss of T cells in the GI during HIV-1 infection. Depletion of T cells can be a direct or indirect effect of the infection: accordingly loss of CD4⁺ T cells may be mediated by direct infection (34), or caused by an immune-mediated clearance of infected cells, or bystander apoptosis (35) through CD95-CD95L dependent mechanism (38), or a combination of these mechanisms.

During the acute phase of HIV or SIV infection opposite to the depletion of CD4⁺ T cells, there is an expansion and/or an influx of CD8⁺ T cells in the GI tract. However, these CD8⁺ T cells fail to clear the virus or prevent virus replication and dissemination during primary HIV-1 infection (125).

In chronic phase: Immune activation in HIV-1 chronic infection is an almost pathognomic feature and one of the strongest predictors of disease progression. Some of the manifestations of the chronic immune activation occurring during HIV-1 infection are increased T cell turnover, increased frequencies of T cells with activated phenotype and increased serum levels of pro-inflammatory cytokines and chemokines (Fig. 3).

In the GI tract, during chronic HIV-1 infection, a depletion of the CD4⁺ T cells continues to occur and this process worsens the damage occurring in the immune system during the acute phase of infection (126). The GI mucosal barrier suffers a serious immunological and structural insult from the very early phases of the infection and this process continues during the chronic phase of HIV-1 infection. Damage to the GI tract during HIV-1 infection may result in microbial translocation. Recently, a study has shown that chronically HIV-1 infected individuals have significantly increased levels of plasma LPS as compare to healthy individuals. Microbial products such as LPS, peptidoglycan and bacterial CpG DNA can directly stimulate the innate immune system through the TLRs or other receptors. The study suggested that microbial translocation may be an important mechanism causing systemic immune activation during chronic HIV-1 infection (28).

In chronic HIV-1 infection, there is a robust and often poly-functional CD8⁺ T cell response which continuously attempts to fight the HIV-1 infection in the GI tract and to control viral replication at certain level. However the CD8⁺ T cells cannot clear the chronic HIV-1 infection (127).

1.5 ANTIRETROVIRAL THERAPY AND IMMUNOTHERAPY

1.5.1 Antiretroviral therapy

ART is considered as the best option for HIV viral suppression and for reduction of morbidity and mortality during HIV infection (128, 129). In spite of the fact that current drugs do not eradicate HIV-1 infection, they help to prolong the relatively healthy life of treated patients as compared to patients who do not receive ART. To date, at least 5 ART drug classes have been recommended to be used for the treatment of HIV infection by the World Health Organization (WHO): Nucleoside reverse transcriptase inhibitors (NRTIs), Nucleotide reverse transcriptase inhibitors (NtRTIs), Non-nucleoside reverse transcriptase inhibitors (NNRTIs), Proteases inhibitors (PIs) and Integrase strand transfer inhibitors (INSTIs) (130). ART is lifelong treatment, and it is currently recommended to use the combination of at least 3 medicines so called highly active antiretroviral therapy (HAART).

Since HIV is genetically highly variable and the patients require lifelong treatment with ART, the major problems with the current therapy are the emergence of drug resistant HIV strains and side effects. In addition, HIV therapy is very expensive and patients under ART treatment need to be monitored regularly and frequently with costly laboratory tests including determination of CD4⁺ T cells counts and viral load, rendering ART not easily accessible to all HIV infected patients in developing countries (131).

Under ART, the viral replications is suppressed to a low level and the peripheral blood CD4⁺ T cell number is increased leading to a certain degree of immune reconstitution. A study in the GI tract showed that ART resulted in the viral load reduction, an increased number of CD4⁺ T cells and a modest reduction in the number of apoptotic cells in the rectal tissue (132). However, CD4⁺ T cell recovery in the tissue was poor and occurred at a much slower rate than the increased of CD4⁺ T cells in peripheral blood (124, 133).

1.5.2 Immunotherapy

In addition to conventional ART, immunotherapy strategies have been investigated to improve immunological recovery during HIV infection. Several approaches have been undertaken which yet have not given a final proof of clinical benefit.

Adoptive therapy: In a adoptive therapy study, infused antigen-specific CTLs showed the capacity to home to sites of virus replication, to retained lytic functions in vivo, and transiently reduce the levels of circulating, productively infected CD4⁺ T cells (134).

IL-2 therapy: IL-2, which is produced by activated T cells, induces proliferation and cytokine production in T cells, B cells, NK cells and T regulatory cells (T reg). IL-2 has been extensively studied in phases I-II trials, with the administration of IL-2 by subcutaneous injection twice/per day. IL-2 led to increased CD4⁺ T cells numbers in HIV-1 infected patients (135-138) and also to a significant increase of the survival of CD4⁺ T memory cells (139). The studies suggest that IL-2 could help in maintaining the functionality of immune cells important for host defense against new antigens as well as for long-term memory to opportunistic infections. To investigate the roles of IL-2 in clinical benefits during HIV-1 infection, two large phase III trials were conducted. The ESPRIT (Evaluation of Subcutaneous Proleukin in a Randomized International Trial) study was conducted in patients with CD4⁺ T cell count > 350 cells/ μ l and the SILCAAT (Subcutaneous, Recombinant, Human IL-2 in HIV-Infected Patients with Low CD4 Counts under Active Antiretroviral Therapy) study in patients with CD4⁺ T cells between 50-299 cells/ μ l. Both studies compared the effect of IL-2 plus ART with ART alone. The primary end-point of both studies was opportunistic disease or death. The CD4⁺ T cell counts were significantly higher in the IL-2 treated group as compared to patients included in the control group. However, no clinical benefit of IL-2 was found in either studies since the increase in CD4⁺ T cells did not reduce the risk of opportunistic infections and death (140).

IL-7 therapy: This cytokine plays an important role in T cell homeostasis, and contributes to T cell development and survival. One prospective open-label, phase I/IIa trial was conducted to investigate the safety and efficacy of IL-7 administered to HIV-1-infected patients treated with HAART. The trial included 13 HIV-1 infected patient under HAART with CD4⁺ T cell counts between 100 and 400 cells/ μ l and plasma HIV-1 RNA levels < 50 copies/ml. Recombinant human (rh) IL-7 was administered (in presence of HAART) with eight subcutaneous injections of two different doses three times a week for a period of 16 days. The rhIL-7 was well tolerated with minor side effects and induced a sustained increase of naive and central memory CD4⁺ and CD8⁺ T cells. The study showed that in lymphopenic HIV-1

infected patients, rhIL-7 therapy induced a substantial quantitative increase in T cells for 48 weeks (141).

In the phase I prospective randomized placebo-controlled study, AIDS clinical trials group (ACTG), a single subcutaneous dose of rhIL-7 was well tolerated with biological activity leading to increased numbers of circulating CD4⁺ and CD8⁺ T cells, predominantly of the central memory phenotype. The number of T reg cells (CD25^{high} CD127^{low}) number was not affected by rhIL-7 therapy (142).

The studies showed that rhIL-7 therapy led to a sustained increase of naive and central memory CD4⁺ and CD8⁺ T cells, and improved T cell function by inducing IFN- γ and/or IL-2 in response to HIV antigen; these results suggest that patients may benefit from intermittent therapy with IL-7 in combination with ART.

IL-7 therapy was well tolerated in HIV-1 infected patients. However, there are some points which need to be considered. Experiments conducted in vitro showed that IL-7 may up-regulate HIV-1 replication(143), and can enhance HIV-1 proviral reactivation (144). A transient level of elevated HIV mRNA was observed in plasma in 4 of 8 patients receiving 10 μ g/kg rhIL-7 dose in (141) and 6 of 11 rhIL-7 treated patients in (142). In this latter study, at day 56, HIV viral load returned to <50copies/ml in all except one patient. In addition, in one study (142) the T reg population was studied; since it was concluded that this population, on the contrary of CD4⁺ T cells, was not expanded the risk remains that development of autoimmunity or other immune dysregulations may accompany IL-7 treatment.

IL-12, IL-10, and IL-15 therapies: IL-12 stimulates T lymphocytes and NK cells to generate a Th1-type immune response. A randomized phase I study was conducted to assess the effect of rhIL-12 therapy in HIV-1 infected patient under HAART. The result showed that IL-12 was well tolerated but had no effect on T lymphocytes subpopulations, antigen specific immune response or viral load (145). The studies on IL-10 and IL-15 therapy also showed no clinical benefit (146, 147).

HIV vaccines: The development of safe prophylactic and therapeutic vaccines with high efficacy is an important goal for the HIV research field. An ideal HIV vaccine should induce cross-neutralizing antibodies against wild-type R5 virus from different clades, strong and broad CD4⁺ T cells and CD8⁺ T cells responses and create long-term memory and mucosal immunity (148). Several approaches have been used for

vaccine development such as using live attenuated, inactivated, virus-like particles, DNA and recombinant vaccines (149). However, despite intensive research, the development of a good candidate vaccine remains elusive. The challenge of developing a HIV vaccine needs new approaches and require new basic research insights (150).

2 AIMS OF THE THESIS

The overall aim of thesis is focused on the regulation of production and role of IL-7 in HIV-1 infection. The specific aims of the thesis include:

1. To analyze the relationship between serum IL-7 concentrations and T cell numbers in HIV-1 infected patients with variable degree of immune dysfunction.
2. To evaluate the role of CD28- T lymphocytes in inflammatory conditions and immune activation through the modulation of DC and T cell responses during HIV-1 infection.
3. To investigate the role of IL-1 β and IFN- γ , cytokines produced during inflammatory conditions, in regulation of IL-7 production by stromal and intestinal epithelial cells.
4. To investigate the mechanism through which high levels of IL-7 lead to up-regulation of CD95 receptor on B cells and increased B cell apoptosis during HIV-1 infection.
5. To analyze the relationship between levels of inflammatory cytokines and IL-7 during HIV-1 infection.

3 MATERIALS AND METHODS

The materials and methods used in the studies enclosed in this thesis are summarized in the following sections.

3.1 PAPER I

Patients: Serum samples and data on T cells numbers were collected for 19 treatment naïve, chronically HIV-1 infected patients in a study period between 13-56 months during 1983-1987 at the Karolinska University Hospital. In addition, 45 HIV-1 infected patients from a previously characterised long-term nonprogressor (LTNP) cohort (151) together with 16 ART-treated, chronically HIV-1 infected individuals from the San Raffaele Institute (Milan) were included in the study. The ethical commissions at the Karolinska Institutet and San Raffaele Institute approved the studies.

Measurement of IL-7 in serum: IL-7 concentration in serum was determined by the Enzyme-linked immunosorbent assay (ELISA) Quantikine high sensitivity immunoassay (R&D Systems, Minneapolis, MN, USA) according to manufacturer's recommendations.

Statistical analysis: Statistical analyses were performed with the Sigmastat program (SPSS Inc., Chicago, IL, USA). Linear regression analysis or Spearman rank order correlation was used to analyse the association and correlation between the variables. IL-7 concentrations in different cohorts were compared using t-test.

3.2 PAPER II

Patients and controls: Blood samples were obtained from 12 HIV-1 infected patients, 9 on combination therapy and 3 treatment naïve. The viral load ranged between <50 and 139 000 copy/ml, and the mean CD4⁺ T cell count was 474 cells/ μ l. Blood was also collected from 8 healthy donors. The ethical commission at the Karolinska Institutet approved the studies.

Cellular studies: The CD28⁻ and CD28⁺ T cell subsets were purified by cell sorter or magnetic separation from peripheral blood. The monocyte-derived DCs were produced by culturing purified monocytes with Granulocyte-macrophage colony-

stimulating factor (GM-CSF) and IL-4 for five days. The cell markers were measured by flow cytometry.

Cell proliferation was assessed as it follows. Naive T cells isolated from healthy individuals were stained with carboxyfluorescein (CFSE) and then cultured at the density of 10^6 /ml in the presence of DCs (10^5 /ml) pre-treated for 24 hours with CD28+, CD28+CCR7-, and CD28- T cells. Proliferation of the CFSE-labeled T cells was analyzed after four days of activation using flow cytometry.

The production of cytokines in cell culture supernatants, including IL-12, IL-10 and TNF, was measured by ELISA.

3.3 PAPER III.

Cell lines and culture conditions: The human colon adenocarcinoma epithelial cell line DLD-1 and the human bone marrow stromal cell line HS27 were cultured in RPMI-1640 medium and Dulbecco's modified Eagle's medium (DMEM; Sigma, St Louis, MO, USA), respectively, with 2 mM L-glutamine, 1% penicillin–streptomycin and 10% heat-inactivated foetal bovine serum (Sigma) in polystyrene flasks with 5% CO₂ at 37 °C. Both cell lines were obtained from the American Type Culture Collection (ATCC).

The cells were seeded in polystyrene 12-well plates (Corning Incorporated, Corning, NY, USA) with medium at a density of 3×10^5 cells/ml for 36 h prior to each experiment. For the experiments, the cells were washed with phosphate-buffered saline (PBS) and fresh medium was added with or without the cytokines: IL-1 β (10 ng/ml), IFN- γ (50 ng/ml), TNF- α (20 ng/ml), IL-2 (10 ng/ml) or the combination of IL-1 β and IFN- γ , was added for 6 h (for mRNA expression) or for 24 h (for protein determination).

Flow cytometric analysis of cytokine and chemokine receptors on DLD-1 and HS27 cells: Cell surface markers of DLD-1 and HS27 cells were investigated by staining the cells with different antibodies and analysis by Flow cytometry; the data was analyzed by WinMDI 2.9 software (Joseph Trotter, La Jolla, CA, USA).

Relative quantification of human IL-7 mRNA in cell lines: Cellular RNA was isolated from DLD-1 and HS27 cell lines using RNeasy Mini Kit (Qiagen, Hilden, Germany). Complementary DNA (cDNA) was synthesized by the Ready-To-Go You-Prime

First-Strand Bead Kit (GE Healthcare Bio-Sciences Corp, NJ, USA) and random primers (Invitrogen, CA, USA).

Relative quantification real-time polymerase chain reaction (PCR) assay was performed by 7900HT ABI PRISM Sequence Detector System (Applied Biosystems) with the human IL-7 assay on-demand kit (catalogue number Hs00174202_m1) and the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) assay (catalogue number: 4333764F) as an endogenous calibrator (Applied Biosystems, Foster City, CA, USA). The relative expression levels of IL-7 mRNA in the cells, previously stimulated with different cytokines, were compared to the average of IL-7 mRNA expression levels on non-stimulated cells and normalized to the GAPDH mRNA expression levels by the $2^{-[\Delta\Delta C(T)]}$ method.

Expression gene profiles using the Affymetrix microarray platform: Total RNA was harvested from the HS27 cell line at 6 h after stimulation with either IL-1 β or IFN- γ , or the combination of both cytokines; non-stimulated cells were used as controls. RNA was harvested from three independent experiments for each type of stimulation (IL-1 β , IFN- γ or both) and controls. HS27 gene expression profiling was performed by using the whole-genome microarray Human Gene 1.0 ST Affymetrix platform (Affymetrix, Inc., Santa Clara, CA, USA) according to standard manufacture's protocols. Image analysis was performed using Affymetrix Command Console (AGCC) v 1.1, and downstream processing was performed with Affymetrix Expression Console (EC) v 1.1.

Measurement of IL-7 and chemokines: IL-7 concentration in the culture supernatant was determined by Quantikine HS high-sensitivity ELISA kit (R&D Systems); the detection range of this cytokine in the supernatant was 0.156–10.0 pg/ml. The levels of CCL5, CCL20 and CXCL11 in the HS27 and DLD-1 culture supernatants were also tested by Quantikine HS high-sensitivity ELISA kits (R&D Systems). All samples were run in duplicate.

Statistical analysis: Statistical analyses were performed with Sigmastat software (SPSS Inc., Chicago, IL, USA). Pearson product-moment correlation coefficient test was used to measure the correlation between IL-7 and other cytokines. Student's *t*-test was used to compare the mean values of IL-7 mRNA expression in cells or IL-7 concentration in culture supernatants between different treatments.

3.4 PAPER IV

Blood collection and cell culture: Blood samples were collected from healthy blood donors and from 51 HIV-1 infected patients, 49 men and 2 women. 31 patients were on combination therapy and 20 were not treated. The ethical committee at the Karolinska Institutet approved the studies. PBMCs were separated by Ficoll gradient centrifugation (Lymphoprep, Oslo, Norway). For cell cultures monocytes, T and B lymphocytes were separated using respectively the CD14 human microbeads, the Pan T cell Isolation Kit and B cells isolation kit II (Miltenyi Biotech, Bergisch Gladbach, Germany). The purity of the selected cell populations was 90-97% as measured by flow cytometry. Cells were cultured at a density of 1×10^6 cells/ml in RPMI-1640 containing L-glutamine, 10% FCS and antibiotics.

Flow cytometric analysis: Flow cytometric analysis of stained cells was performed by using a FACS LSR II (Becton Dickinson, San Diego, CA) and the data were analysed with FlowJo v. 8.4.4 software (Tree Star Inc., Ashland, OR).

Apoptosis detection: CD95 mediated apoptosis was triggered by human recombinant CD95 ligand (CD95L) (1mg/ml), cross-linked with 20 mg/ml anti-His antibody (both from R&D System, Minneapolis, MN). FITC-conjugated Annexin V reagent (BD Pharmingen) was used to measure apoptosis according to manufacturer's instructions. The fractions of cells stained as Vivid negative-Annexin V positive CD3 positive (T lymphocytes), or CD19 positive (B lymphocytes) were considered as apoptotic cells.

Protein Array: Sorted B cells were incubated in IL-7 treated or non treated T cell supernatants for 30 minutes and the phosphorylation patterns was determined using the Human Phospho-Kinase Array Kit (R&D Systems) according to manufacturer's instruction.

Detection of IFN- γ mRNA levels: IFN- γ mRNA levels in T cells were detected by real-time PCR with a 7900 CD95T ABI PRISM Sequence Detector System (Applied Biosystem).

Measurement of cytokine concentrations: IFN- γ in culture supernatants was measured by ELISA (BD Pharmingen). Level of IFN- γ , IL-7 and IL-2 in HIV-1 plasma samples were quantified by Luminex technique with Milliplex[®] Map kit, High Sensitivity

Human Cytokine Immunoassay (Millipore Corporation, 290 Concord Road, Billerica, MA 01821, USA).

Statistical analysis: Statistical analysis was performed using the Prism (version 5.0a for Mac OS X, GraphPad Software, La Jolla, CA) using t test and Spearman test.

3.5 CORRELATION OF IL-7 WITH INFLAMMATORY CYTOKINES IN HIV-1 INFECTED PATIENTS RECEIVING ART THERAPY IN VIETNAM (UNPUBLISHED RESULTS)

Sample collection: Blood samples were collected from 18 HIV-1 infected individuals, classified as AIDS. at the Tayho District Health Center, Hanoi, Vietnam. Among these patients 11 were males and 7 females; the mean age was 32 years. After obtaining written informed consent, blood was collected from all patients at six time points during 1-year period corresponding to start of treatment and 2 weeks, 1, 3, 6 and 12 months after the start of the treatment. Blood samples from 24 healthy HIV negative individuals, age and sex matched, were also collected. The Hanoi Medical University Review board in Bio-medical Research Ethic approved the study.

CD4⁺ T cell count: CD4⁺ T cells were measured by BD FACScount (Becton Dickinson, USA) in all fresh blood samples.

HIV-1 viral load: HIV-1 viral load was determined in plasma by the COBAS TaqMan HIV-1 Test (ROCHE Molecular Systems, Inc., Branchburg, NJ, 08876 USA).

Measurement of cytokine concentrations: The concentration of IL-1 β , IL-2, IL-7 and IFN- γ were simultaneous quantified in plasma samples by Luminex technique with Milliplex[®] Map kit, High Sensitivity Human Cytokine Immunoassay (Millipore Corporation, 290 Concord Road, Billerica, MA 01821, USA).

Statistical analyses: Statistical analysis was performed with Sigmastat software (SPSS Inc., Chicago, IL, USA) using Pearson product-moment correlation coefficient test, ANOVA on ranks and t test.

4 RESULTS AND DISCUSSION

4.1 RELATIONSHIP BETWEEN IL-7 LEVELS AND T CELL COUNTS IN HIV-1 INFECTED PATIENTS (PAPER I)

It has been shown that during HIV-1 infection a negative correlation exists between high levels of IL-7 and CD4⁺ T cell counts in blood (94, 112, 113). In this lymphopenic condition the high IL-7 levels can be a potent factor to promote the recovery of T cells through increased survival and proliferation. However it is still not fully understood what exactly regulates the IL-7 production and the roles of IL-7 in controlling homeostasis of T lymphocytes during HIV-1 infection. We got the opportunity to analyze longitudinal samples collected at multiple time points from HIV-1 infected individuals naïve to treatment, and specimens from ART- treated patients and LTNPs (paper I). We investigated the IL-7 levels in relation to different subgroups of T cells in HIV-1 infected patients with different degree of immunodeficiency.

In the treatment naïve HIV-1 infected individuals, we measured the IL-7 levels at 2-4 time points during a period of 13-56 months and observed a correlation of IL-7 with different groups of T cells. In this study group, we found no correlation between IL-7 levels and CD4⁺ T cells, but we observed a statistically significant negative correlation between IL-7 and CD8⁺ T cells and CD3⁺ T cells. This indicated a potential role of T cell lymphopenia in increased serum IL-7 levels in ART naïve individuals. However, we did not observed any correlation between the changes of T cell numbers during the study period with the IL-7 levels. This finding may indicate that during the late phase of HIV-1 infection high levels of IL-7 may not be efficient to stimulate the increase of T cell numbers.

We analysed samples collected at different time points from chronic HIV-1 infected patients naïve to treatment in order to assess the changes of IL-7 levels in relation to the numbers of CD4⁺ T and CD8⁺ T cells over time. We found a wide variation in the correlation of IL-7 with CD4⁺ and CD8⁺ T cells among the patients that may reflex individual differences. This suggests that IL-7 levels may play a role in certain patients during chronic HIV-1 infection, perhaps only in those patients whose immune system is yet not exhausted and is able to respond to IL-7 for T cell homeostasis.

A recent study showed that HIV-1 infection led to collagen deposition and fibrosis which restricted T cell access to the survival factor IL-7 on the fibroblast reticular cells (FRC), a source of IL-7 production, resulting in apoptosis and depletion of T cells (46) . In turn, depletion of T cells led to a reduced production of lymphotoxin- β , a survival factor for FRCs. These events thus create a vicious cycle of the depletion T cells and damage the FRC network. These new findings indicate that IL-7 may still have a positive role in regulating T cells homeostasis when fibrosis has yet was not occurred in FRC; thereafter IL-7 producing cells are damaged by collagen deposition. The level of CD4+ T cell reconstitution may therefore also be dependent on the level of fibrosis of FRC which in turn affects the level of IL-7 production.

We compared the IL-7 levels in plasma obtained from LTNP patients, characterized by CD4+ T cells counts of at least 500cells/ μ l, viral control for 7-10 years without ART, asymptomatic HIV-1 infection and good health condition, with the levels found in chronic HIV-1 patients under ART. There was no difference for the IL-7 levels between the two groups. We also compared the IL-7 levels of 20 patients who lost their LTNP status during a 3 year follow-up period and where the CD4+ T cell number declined under 500cells/ μ l and the viral load increased with patients with stable LTNP status. Interestingly, the IL-7 levels were significantly lower in the patients with stable LTNP status than in patients who lost their LTNP status during the follow-up period (Fig. 4). This finding can be a valuable biological marker to predict the disease progression in HIV-1 infected LTNPs.

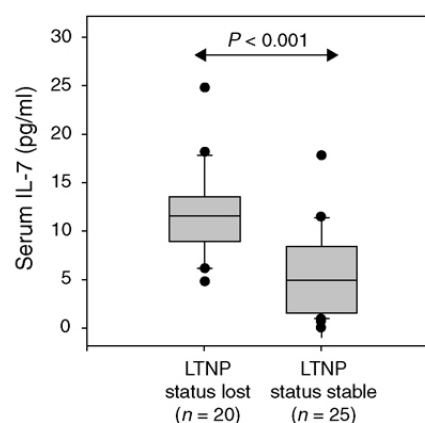


Figure 4. Serum IL-7 level in LTNP patients who lost their LTNP status and stable LTNP patients. IL-7 concentration was measured and compared between the two groups of patients.

As introduced above, IL-7 is an important survival and proliferation factor for T cells; accordingly, when the IL-7 level is high, the T cells number should likely be maintained stable. However it is remarkable that in those patients who lost the LTNP status the CD4+ T cell number declined in spite of the fact that IL-7 levels were high. It is possible that high levels of IL-7 could act to induce viral replication in the viral reservoirs in HIV-1 infected individuals (143, 144). Some explanations for clinical progression from the LTNP status have been previously discussed, including aberrant activation of the immune system (152).

In conclusion, this study presented in paper I showed that in chronic HIV-1 infection, increased IL-7 concentration is generally observed in parallel with T cell depletion. An increase of IL-7 level in LTNPs may be predictive of disease progression.

4.2 POTENTIAL ROLE OF CD8+CD28-T CELLS IN IMMUNE ACTIVATION DURING HIV-1 INFECTION (PAPER II)

CD28 is a cell membrane receptor which is expressed on most of the CD4+ T cells and CD8+ T cells in human peripheral blood. CD28 acts as co-stimulatory molecule, which is required for T cell activation when binding to its ligand B7.1 (CD80) or B7.2 (CD86) on activated APCs (153). CD28 is down-regulated upon T cells activation. Loss of CD28 on T cells is the most consistent factor related to decline of immune responses during aging in humans. It has been shown that at birth almost all human T cells express CD28; by the age 80, on the contrary, about 10-15% of peripheral blood CD4+ T and 50-60% of CD8+ T cells lack CD28 expression (154). Besides, the increase of CD28- T cells population has also been associated with persistent inflammatory conditions such as rheumatoid arthritis(155), multiple sclerosis (156) and Wegener's granulomatosis (157).

The CD28- T cells population is expanded during the course of HIV-1 infection, representing between 50-80% of the peripheral T cell pool, mostly CD8+ T cells (158-160). It has been shown that the increase of the CD8+CD28- T cells is associated with the suppressor function of this sub-set of T cells during HIV-1 infection (161, 162). The suppressor T cells exert their immunosuppressive effects on T cell activation by modulating the T cell activating potential of DCs or by directly affecting activated T cells through soluble mediators (161, 163). In this study (paper II) we aimed at

investigating whether the increased of the CD28- T cell population observed during HIV-1 infection could influence DC functions and thus contribute to immunodeficiency occurring during the course of HIV-1 infection.

CD28+ and CD28- isolated from HIV-1 infected and healthy individuals were co-cultured with monocyte-derived DCs for 24 hours. After that DCs were stimulated with LPS for another 24 hours and thereafter the DC's activation markers HLA-DQ, CD86 and CD83 were measured (Fig. 5). Our data showed that, either CD28+ or CD28- T cells were able to induce a mature DC phenotype by inducing up-regulation of those three activation molecules. Interestingly, CD28- T cells induced the same level of activation on DCs as CD28+ T cells. In addition, neither of those T cell populations was able to inhibit LPS- induced maturation of DCs.

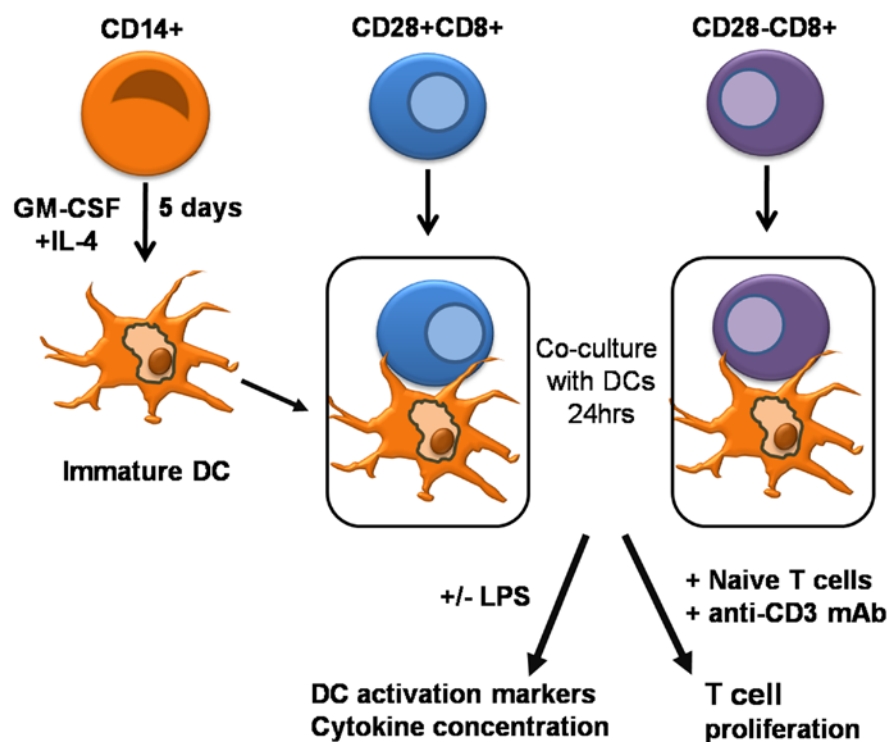


Figure 5. Outline of the experimental design for the study.

We also measured the production of pro-inflammatory cytokines by DCs and found that the presence of CD28- T cells, instead of having an inhibitory effect, strongly increased IL-12 and TNF production by DCs induced by LPS. The production of the anti-inflammatory cytokine IL-10 was not changed by the presence of any of the T cell populations.

We next evaluated whether CD28-T cells purified from HIV-1 infected and healthy individuals were able to affect the proliferation of naive T cells triggered by DCs and anti-CD3. Purified CD28- T cells, CD28+ T cells and CD28+CCR7- effector memory T cells were cultured with DCs for 24 hours (Fig. 5). Thereafter isolated naive T cells were added to cultures and stimulated by anti-CD3 mAbs. All three T cell subsets pre-incubated with DCs slightly affected the proliferation of the third party T cells. We found that there was a slight reduction in the number of proliferating T cells when DCs were cultured in presence of CD28- T cells and of CD28+CCR7- memory cells as compared to CD28+ T cells, possibly reflecting negative feedback functions created by antigen-experienced T cells to limit further T cell activation. Interestingly, the proliferation of third party T cells was equally observed in both HIV-1 infected and healthy individuals.

In contrast to the immune suppressive functions of in-vitro generated CD8+CD28- T cells shown in previous publications (161, 163), our results indicated that naturally occurring CD28- cells do not show suppressor functions but these cells contribute to increased DC activation. In conclusion, our study showed that the accumulation of CD28- T cells during HIV-1 infection may not lead to DC or T cell suppression but that this population may contribute to accelerated inflammatory reactions and immune activation through increased production of inflammatory cytokines by DCs. It is of interest to point out that CD28- T cells are expanded during HIV-1 infection although they have a low level of expression of the IL-7R α (94).

4.3 REGULATION OF IL-7 PRODUCTION BY PROINFLAMMATORY CYTOKINES (PAPER III)

As mentioned above, IL-7 is an essential cytokine in T cell homeostasis and for survival and proliferation of T cells especially in lymphopenic conditions. IL-1 β , a highly active proinflammatory cytokine, is efficiently produced by macrophages upon LPS-mediated TLR4 stimulation (164). In HIV-1 infection, IL-1 β levels were shown to increase during primary infection to decline to undetectable levels during the chronic stage of infection (165). Single strain RNA (ssRNA) HIV genomes, which can be considered as pathogen-associated molecular patterns (PAMPs), can activate the inflammasome leading to IL-1 β production from macrophages and DCs as part of innate immune responses (166, 167). A published study has shown that HIV derived

ssRNA is recognized from TLR7 and TLR8 and stimulates macrophages and DCs to secrete proinflammatory cytokines (168); the production of IL-1 β upon these conditions has not been assessed. In addition, the HIV-1 glycoprotein gp120 has been shown to induce IL-1 β release from macrophages in vitro (169). During the early stage of HIV-1 infection, HIV-1 causes a strong damage to the GI tract structure and intestinal epithelial barrier; there is a massive depletion of T lymphocytes and a high number of infected CD4⁺ T cells in GI. Recently, a study showed that the intestine, which surface is the largest in the body, is the major source for IL-7 production in vivo (84).

In addition, IFN- γ , which is present in the human mucosa during inflammation (170), has also been reported to have a regulatory role in IL-7 production (80). Different cell types are known to produce IL-7 of which bone marrow stromal cells and intestinal epithelial cells are among the main sources of IL-7 production. However, the mechanisms and cellular factors regulating IL-7 production are still unclear. Therefore, we investigated whether IL-1 β and IFN- γ regulate IL-7 production by intestinal epithelial and bone marrow stromal cells (paper III). In a setting in which damage of the epithelial barrier leads to the compromised homeostasis of immune cells, the modulation of IL-7 levels during HIV-1 infection may impact on survival of T cells in the gut.

The presence of IFN- γ in culture consistently upregulated the production of IL-7 from stromal and epithelial cells (Fig. 6). To investigate the impact of IL-1 β on the expression of IL-7 mRNA induced by IFN- γ , we stimulated the cells with the combination of both cytokines. We found that IL-1 β was able to down-regulate IL-7 mRNA expression in both DLD-1 ($P = 0.006$) and HS27 cells ($P < 0.001$). Moreover, for the HS27 cells, IL-1 β completely abrogated the positive effect of IFN- γ on IL-7 mRNA expression (Fig. 6a,b). Of relevance is that IL-1 β induced a consistent down-regulation of IL-7 in the HS27 cells in the range of 1–100 ng/ml, with a maximum effect reached already at 1 ng/ml (Fig. 7).

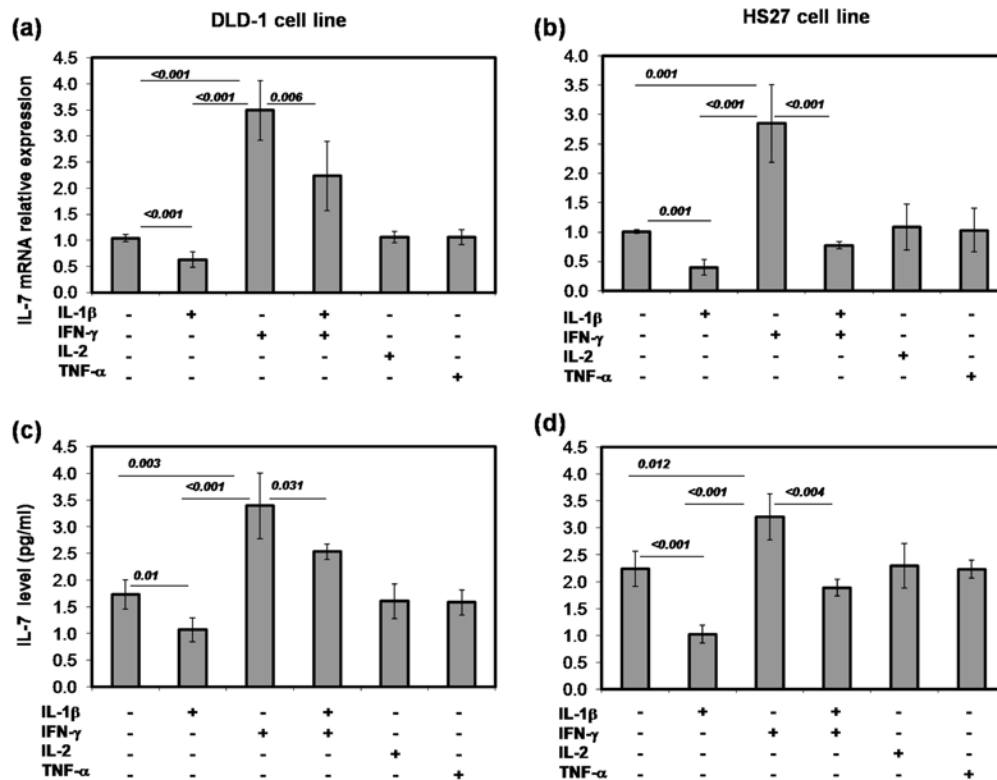


Figure 6. Regulation of IL-7 production at mRNA and protein level by IL-1 β and IFN- γ in DLD-1 and HS27 cells. (a) and (b) Relative expression of IL-7 mRNA measured by real-time PCR in DLD-1 and HS27 cells with and without treatment with different cytokines for 6 h. (c) and (d) IL-7 protein levels measured by quantitative ELISA in culture supernatants of DLD-1 and HS27 cells with different cytokines and in control cultures at 24 h. The results represent the mean values and standard deviation of four different experiments.

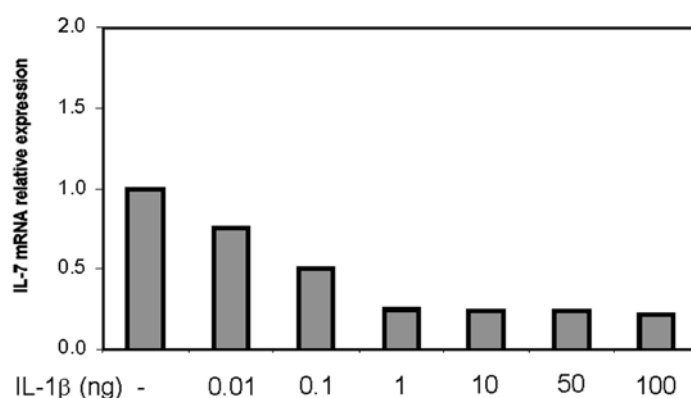


Figure 7. The effect of different concentrations of IL-1 β on the regulation of IL-7 mRNA expression in HS27 cells. Relative expression of IL-7 mRNA was measured by real-time PCR in HS27 cell line exposed to different IL-1 β concentrations.

The IL-7 concentration of supernatants collected at 6 and 24 h from cells treated with IL-1 β , IFN- γ , IL-2, TNF- α and the combination of IL-1 β and IFN- γ , were tested by quantitative ELISA (Fig. 6c,d). At 6 h, IL-7 was not detectable in any of the supernatants of the cytokine-treated or control cells (data not shown). However, at 24 h, both cell lines spontaneously produced a measurable amount of IL-7 protein, with a higher concentration per cell number in HS27 cells compared to DLD-1 cells (2.2 pg/ml and 1.8 pg/ml per 10⁶ cells, respectively). Of note, the patterns of IL-7 protein levels in the supernatants from the treated cultures were similar to those of the mRNA levels. Treatment with IFN- γ and IL-1 β significantly enhanced (DLD-1, $P = 0.003$; HS27, $P = 0.012$) or reduced (DLD-1, $P = 0.01$; HS27, $P < 0.001$), respectively, the IL-7 production in both cell types. In addition, treatment with the combination of IL-1 β and IFN- γ caused a significant reduction in IL-7 protein production, in cultures of either cell types, compared to IFN- γ -treated cultures. This effect of IL-1 β was more pronounced in HS27 cells than in DLD-1 cells (Fig. 6c,d). Stimulation with IL-2 and TNF- α had no effect on IL-7 production.

To investigate whether treatment of HS27 cells with IL-1 β and/or IFN- γ could lead to changes in the expression of genes important for regulation of immune responses, we derived a gene expression profile of the stromal HS27 cell line (treatment with IL-1 β and/or IFN- γ , or no treatment) by microarray analysis using the whole-genome microarray Human Gene 1.0 ST available in the Affymetrix platform. For each gene and treatment group an average value of expression was derived from the 12 samples analysed, including control cell cultures ($n = 3$) and cultures treated with IFN- γ ($n = 3$), IL-1 β ($n = 3$) and the combination of the two cytokines ($n = 3$) (Fig. 8).

One interesting aspect of the biology of epithelial and stromal cells in primary and secondary lymphoid organs is their capacity to produce chemokines which regulate the recruitment of immune cells into the tissue. For that reason, we analysed the gene profile of chemokines relevant for T cell and neutrophil migration. It is interesting that of the 18 genes for chemokines presented in Fig. 9 and included in the Affymetrix platform, 14 were dysregulated by the presence of either IL-1 β or IFN- γ , or the combinations of these two cytokines. For the CCL8, CCL20, CXCL9, CXCL10 and CXCL11 genes, the expression was increased more than 500-fold. We confirmed by ELISA that the treatment of DLD-1 and HS27 cells with IL-1 β or IFN- γ , or the combination of IL-1 β and IFN- γ , induced the production of the chemokines CCL5,

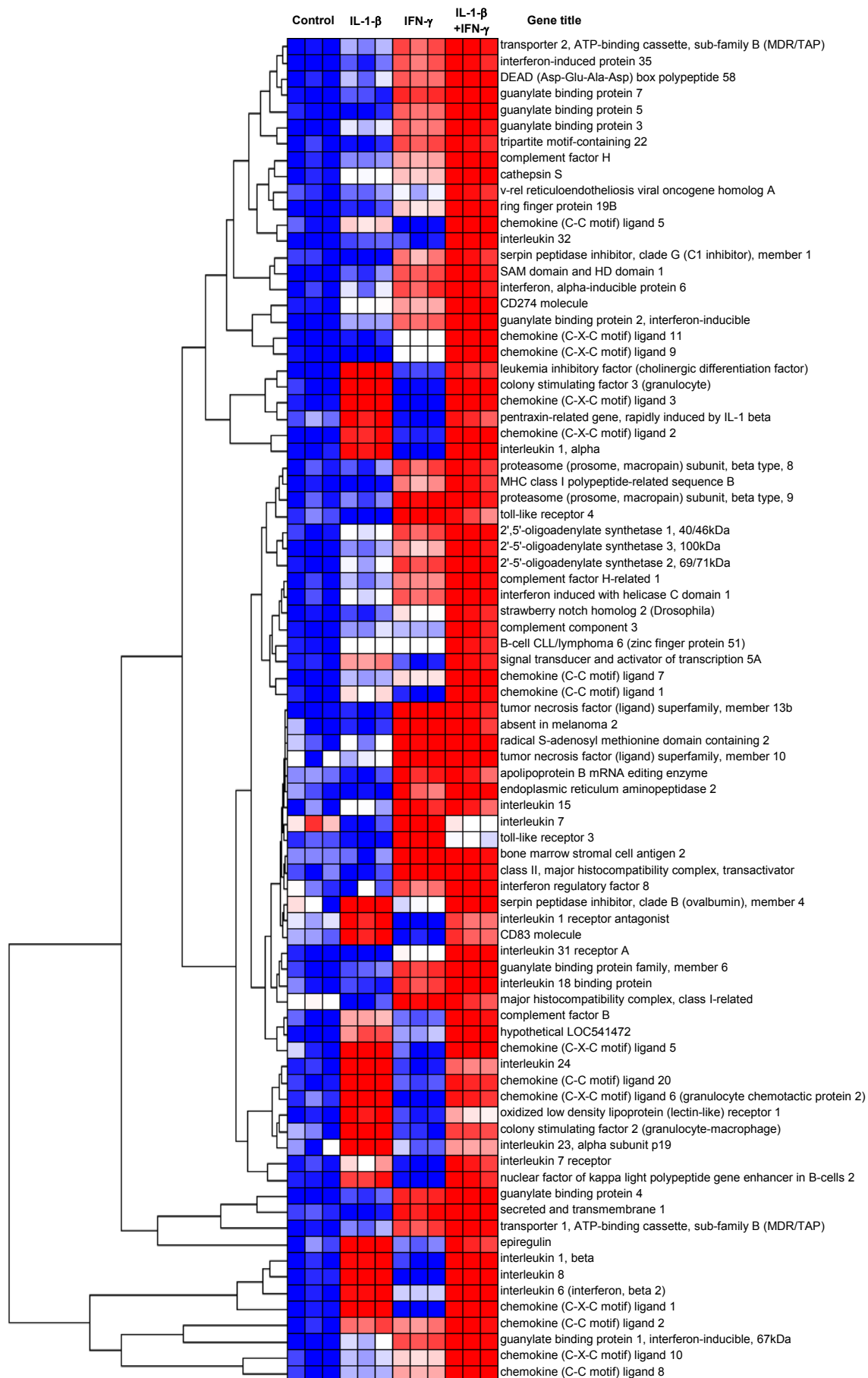


Figure 8. Microarray profiles of immune response genes altered upon treatment of HS27 cell line with IL-1 β , IFN- γ , or the combination of the two cytokines as compared to control HS27 cells without any stimulation. Heat map of the immune response genes, which are up- and down-regulated upon different treatments. Only the genes that display significant changes between any of two groups in *t*-test with $p \leq 0.0005$ with fold change ≥ 4 or ≤ -4 are shown. The color from red to blue represents from high to low gene expression level, respectively. Each column represents data from an independent culture; the control culture and each type of treatment was run from the mRNA of 3 independent cultures.

CCL20 and CXCL11 in culture supernatants. These are three important chemokines that regulate the homing of T cells to lymphoid tissues, and in spite of the different amount of chemokines produced by the individual cell lines, the cumulative effect of production of these chemokines may be T cell chemoattraction.

It has been shown that during acute HIV-1 infection there is a consistent increase in the expression of both IL-1 β and TNF in the GALT and peripheral lymphoid tissue. The expression of IFN- γ also increased significantly in these tissues (171). Another study showed that translocation of microbial components, occurring through the gut epithelium during HIV-1 infection (28), may lead to production of IL-1 β and IFN- γ by macrophages stimulated through the TLRs. In addition, increased levels of HIV-1 ssRNA in viremic patients can also stimulate directly macrophages and DCs to produce inflammatory cytokines through PAMP receptors (166-168). In this setting, our findings of the effect of IL-1 β and IFN- γ on the production of IL-7 by epithelial and stromal cells may contribute to the understanding of pathological events of CD4 $^{+}$ T cell depletion in lymphoid tissues during HIV-1 infection. IL-1 β , released from activated macrophages and DCs as result of the inflammatory process in the gut, may lead to reduced IL-7 production by epithelial cells locally; in turn low level of IL-7 affect the survival of T cells present in this environment.

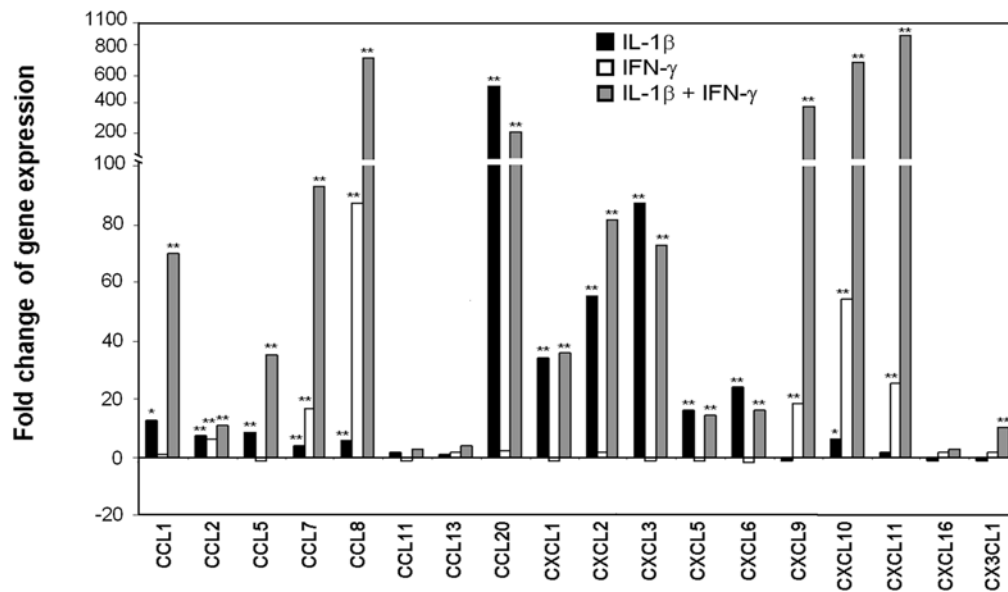


Figure 9. Microarray gene expression profile of chemokines in HS27 cells. The microarray gene expression profiles of chemokines changed upon treatment of HS27 cells with IL-1 β and/or IFN- γ , as compared to control cells without stimulation at 6 h. ** $P < 0.001$; * $P < 0.01$.

During the early stages of HIV-1 infection, CD8⁺ T cell counts were found to be increased in the GALT (171). The CD8⁺ T cells are probably recruited to the GALT in order to eliminate HIV-1 infection. In our study, we examined whether the pro-inflammatory cytokines IL-1 β and IFN- γ could stimulate stromal and epithelial cells to alter the production of factors important for chemoattraction of T cells, mimicking a process that may take place in the inflamed intestinal mucosa. We found that there was an upregulated production of several chemokines important for mobility of T cells and some other immune cells by HS27 and DLD-1 cells treated with either IL-1 β or IFN- γ . This suggests that the production of IL-1 β and IFN- γ in the gut by macrophages activated by HIV-1 or microbial components may be directly involved in the homing of CD8⁺ T cells to the gut mucosa to control the infection.

One of the important findings in our study is that IL-1 β significantly down-regulated IL-7 production in stromal and intestinal cells. If these findings reflect what happens in vivo during HIV-1 infection in the gut, the possibility exists that low IL-7 level may contribute to poor survival of CD4⁺ T cells in the gut inflammatory environment also increased by active HIV-1 replication.

4.4 THE IMPACT OF INFLAMMATORY CYTOKINES ON THE EXPRESSION OF SURVIVAL FACTORS FOR PLASMA CELLS (PAPER III AND UNPUBLISHED RESULTS)

One aspect on the work conducted on the exposure of stromal cells to inflammatory cytokines is how the presence of IL-1 β and IFN- γ may contribute to the change of the expression of cytokines and factors important for the survival of plasma cells (PCs).

PCs reside in the bone marrow (BM) next to reticular stromal cells, which form a survival niche for the PCs and ensure long-term survival of PCs (172). In the special microenvironment provided by reticular stromal cells, PCs produce a large amount of antibodies. The control of PCs survival in niches in the BM is under the control of survival factors produced from stromal cells which are not completely defined in number and in relevance (173).

A population of stromal cells defined as VCAM1+ CXCL12+ -cells have been described to provide survival signals to long lived PCs within the BM and to represent approximately 1% of stromal cells (174). Other characterized factors which have been involved in the survival of plasma cells include IL-4, IL-5, IL-6, IL-10, IL-21, the factors BAFF and APRIL, and the adhesion molecules Inter-cellular adhesion molecule 1 (ICAM-1) and Vascular cell adhesion protein 1 (VCAM-1) (172, 173).

We analyze the microarray results obtained from the stromal cells cultured in presence or absence of IL-1 β and IFN- γ in relation to survival factors for stromal cells (paper III). The results are summarized in table 1. Interestingly, the expression of several of the factors reported to be important for the survival of plasma cells is altered after exposure to IL-1 β and IFN- γ . For some of the factors like IL-6, BAFF, ICAM-1 and VCAM-1 the gene expression was significantly up-regulated more than 10 times. These findings suggest that the result of an inflammatory environment in the BM, but also at other anatomical sites where survival of B cells may be dependent on stromal cells, e.g. the gut, may lead to a profound deregulation of the expression of survival factors for PCs. The results presented in the table should be verified with other methods, before conclusions can be reached. Also of interest is to culture PCs generated in vitro together with stromal cells exposed to the action of IL-1 β and IFN- γ to verify how the conditions created in vitro alter the PCs survival.

Recent data indicate that memory CD4⁺ T cells are also maintained in the BM (172). In the BM the specific T lymphocytes resided in contact with IL-7-expressing, VCAM1⁺ stromal cells, which also comprise about 1% of BM cells. The IL-7-expressing, VCAM1⁺ stromal cells are a distinct population from VCAM1⁺ CXCL12⁺ stromal cells.

	IL-1 β	IFN- γ	IL-1 β +IFN- γ
CXCL12	-1,64*	1,06	-1,21
IL-4	-1,11	-1,51*	-1,53*
IL-5	1,02	-2,10*	-2,35**
IL-6	23,44**	2,99**	26,29**
IL-10	-1,12	-1,09	-1,09
IL-21	-1,10	1,04	1,11
APRIL	-1,18	1,01	-1,05
BAFF	1,16	15,31**	12,89**
ICAM-1	11,25**	10,62**	43,93**
VCAM-1	3,18*	1,28	32,15**

*Table 1. Gene expression of factors relevant to plasma cell survivals which are produced from stromal cells exposed to IL-1 β and IFN- γ . The gene expression profiles of factors relevant to plasma cell survivals changed upon treatment of HS27 cells with IL-1 β and/or IFN- γ , as compared to control cells without stimulation at 6 h. *p<0.05; **p<0.005.*

4.5 ROLE OF IL-7 IN PROMOTING CD95-INDUCED APOPTOSIS OF B CELLS IN HIV-1 INFECTION (PAPER IV)

During HIV-1 infection, in addition to the impaired function of T lymphocytes, several severe dysfunctions occur to B cells leading to profound defects in the humoral immune system. One parameter of B cells dysfunctions is the decreased survival of

activated B cells in HIV-1 infected individuals which has been previously associated with the increased expression of CD95- a receptor of TNF super-family (175) .

A study previously conducted from our group showed that the exposure of T cells to IL-7 in culture lead to up-regulation of CD95 expression and priming of T cells for CD95 mediated apoptosis in vitro. The potential role of IL-7 in up-regulation of CD95 was verified in vivo in IL-7 treated macaques and in HIV-1 infected patients through the positive correlation of IL-7 levels and CD95 expression on T cells (176). During HIV-1 infection the accumulation of immature, circulating transitional B cells has been associated with high IL-7 levels indicating a potential effect of IL-7 on B cell homeostasis (177, 178)

The aim of the study presented in paper IV was to investigate the mechanism leading to increased apoptosis of B cells during HIV-1 infection, a chronic infection often associated to high circulating IL-7 levels. We cultured PBMCs in presence of IL-7 and interestingly, upon these conditions, B cells up-regulated CD95 expression at high level, comparable to the upregulation of CD95 previously reported to occur on T cells (176). Moreover, we analyzed CD95 expression on different B cell subsets and found that all subsets of B cells showed comparable levels of CD95 up-regulation when PBMCs were treated with IL-7 at 25ng and 2.5ng/ml for 5 days.

Since mature B cells do not express the IL-7R α , IL-7 induced CD95 expression on B cells is likely to occur through an indirect mechanism. The up-regulation of CD95 did not occur on B cells which had been directly co-cultured with IL-7 for 3 days. But B cells up-regulated CD95 expression in the presence of IL-7 treated T cells, either in co-culture or when they were grown in a trans-well system, or when IL-7 treated T cell supernatant was added to B cells. All these different conditions lead to a similar level of CD95 up-regulation on B cell suggesting that IL-7 induces up-regulation of CD95 expression on B cells through a soluble factor released from T cells.

Next we investigated whether the up-regulation of CD95 expression on B cells induced by IL-7 had an effect on B cell survival when B cells were exposed to recombinant CD95L. PBMCs were cultured in the presence or absence of IL-7 for five days and CD95 expression and sensitivity to CD95L induced apoptosis of both T and B lymphocytes was monitored at different time points. As already shown for T cells, the increase of IL-7 induced CD95 expression on B cells resulted in the increased

sensitivity to CD95 mediated apoptosis at day 5 of culture, as compared to control cells. These results indicated that the increase in CD95 expression on B cells, mediated by IL-7, lead to a higher sensitivity of B cells to CD95 mediated apoptosis, suggesting a potential link between lymphopenic conditions and B cell apoptosis during HIV-1 infection.

In order to identify the factor which is released from IL-7 treated T cells and which leads to CD95 up-regulation on B cells, we next investigated signaling pathways induced in B cells by IL-7 treated T cell supernatant by protein array. This array allows the detection of 46 phosphorylation events linked to different signaling components. Purified B cells were incubated in IL-7 treated or non-treated T cell supernatants for 30 minutes and thereafter B cell phosphorylation patterns compared. The results showed that STAT1 phosphorylation on residue Y710 signal was increased in response to the IL-7 treated T cell supernatant. To verify the results illustrated above, Fluudarabine, a STAT1 inhibitor, was used in culture. The presence of Fluudarabine led to a considerable reduction in the up-regulation of CD95 on B cells in presence of IL-7 treated T cell supernatant. The results strongly suggested that STAT1 serves as the signaling mediator of IL-7 induced effects on B cells.

Phosphorylation of STAT1 occurs in response to both type I and II IFNs where type I IFN induces signaling through the STAT1/STAT-2 heterodimers, and IFN- γ mediates its effects by inducing STAT1 homodimers (179, 180). In our experiments we did not detect phosphorylation of STAT-2 or other STATs molecules by protein array suggesting IFN- γ as a possible mediator of IL-7 effects on B cells. Moreover, it has been previously shown that IFN- γ can induce CD95 expression on some transformed cell lines (181, 182). In order to verify the involvement of IFN- γ in mediating up-regulation of CD95 expression on B cells, we measured the IFN- γ concentrations in supernatants from T cells cultured in presence or absence of IL-7. In line with our hypothesis, IL-7 treatment induced the secretion of high levels of IFN- γ by T cells, and the IFN- γ production remained high during 11 days of culture. The IFN- γ production by IL-7 treated T cells was also verified by IFN- γ intracellular staining in IL-7 treated T cells, and by real-time PCR for detection of IFN- γ mRNA. We also cultured B cells with IL-7 treated T cell supernatants in presence or absence of IFN- γ neutralizing antibodies and the result showed that IFN- γ neutralization efficiently blocked the ability of IL-7 treated T cell supernatants to induce STAT1 activation and CD95 up-regulation in B cells. These findings showed that IFN- γ , induced by IL-7 treatment of T cells led

to up-regulation of CD95 expression on B cells and also increased their sensitivity to CD95 mediated apoptosis.

Our data showed that the production of IFN- γ by T cell is dependent on IL-7 concentration. It has been shown that stromal cells are able to concentrate this cytokine on their cell surface and to deliver it to IL-7 sensitive cells; these properties of stromal cells may also have an impact on the efficiency of IL-7 delivery to target cells (183, 184) since stromal cell may be able to concentrate low IL-7 concentration in order to assist T cells. We tested whether the IL-7 induced IFN- γ production from T cells can be further augmented by cell types that present IL-7 on their cell surface. The HS27 cell line or human monocytes were cultured in 96-well plates and pretreated with different concentrations of IL-7 for 2 hours at room temperature; thereafter, purified T cells were added to the cultures for 3 days. IFN- γ production was measured by ELISA after 3 days of culture. The results showed that the presence of the HS27 cell line had a strong effect on the IL-7 induced IFN- γ production in T cells. Monocytes, although less efficiently than HS27, could also increase IFN- γ production induced by IL-7. These results showed that stromal cells surrounding T lymphocytes in the various tissues may also have a strong impact on IL-7 efficiency.

To verify the potential role of IL-7 in regulation of B cell survival in lymphopenic conditions, we studied the expression of CD95 in B cells and the IFN- γ and IL-7 serum levels in a Swedish cohort of 51 HIV-1 infected patients. Interestingly, the result showed a strong positive correlation between IL-7 and IFN- γ concentrations in the serum ($p < 0.0001$, $r = 0.68$) demonstrating the potential effect of IL-7 on IFN- γ production from T cells in HIV-1 infected individuals. In addition, the concentrations of IL-7 and IFN- γ were significantly and positively correlated with CD95 expression on B cells ($p < 0.01$ and $p < 0.02$ respectively). Taken together, the results suggest a potential role of IL-7 in priming B cells to apoptosis via up-regulation of CD95 expression induced through the IFN- γ cytokine in HIV-1 infected individuals.

High levels of IL-7 are usually observed in HIV-1 induced lymphopenic condition (94, 113). In this study we showed that IL-7 stimulated T cells to produce an increased level of IFN- γ , which in turn upregulated CD95 expression on B cells. CD95-expressing B cells are sensitive to apoptosis. In addition, we previously showed that IFN- γ is an inducer of IL-7 production in stromal cells (185), one of the main cellular sources for IL-7 production. Thus high level of IFN- γ could stimulate stromal cells to produce an

elevated level of IL-7. These events can thus create a vicious cycle ultimately leading to B cell apoptosis; this mechanism may possibly be operating during HIV-1 infection (Fig. 10).

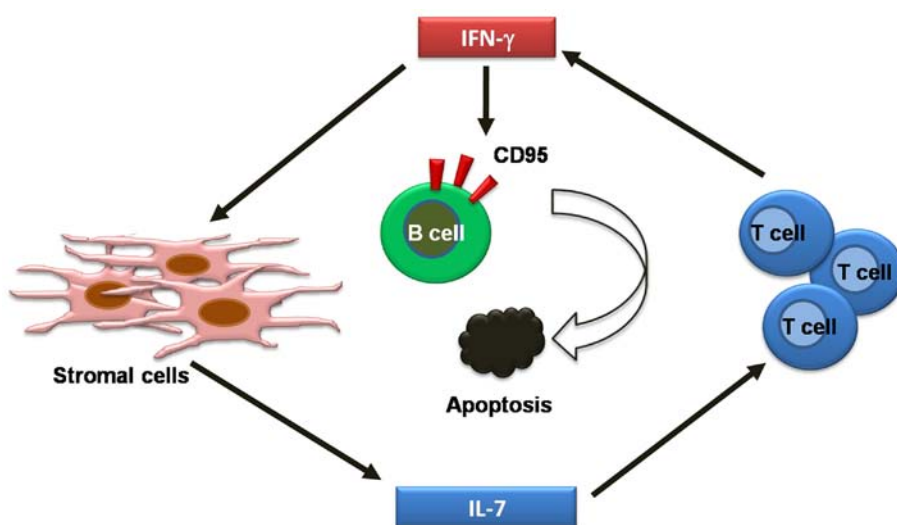


Figure 10. Possible mechanism leading to B cell apoptosis in HIV-1 infection. In HIV-1 infection, high level of IL-7 stimulates T cells to produce an elevated level of IFN- γ . In turn IFN- γ induces B cell apoptosis through increased CD95 expression. High level of IFN- γ also stimulates stromal cells to increase IL-7 production.

4.6 CORRELATION OF IL-7 WITH INFLAMMATORY CYTOKINES IN HIV-1 INFECTED PATIENTS RECEIVING ART THERAPY IN VIETNAM (UNPUBLISHED RESULTS)

In paper III we have shown that IL-7 expression was regulated by INF- γ and IL-1 β . It was of interest to investigate the correlation of inflammatory cytokines INF- γ and IL-1 β with IL-7 and IL-2 in biological specimens from HIV-1 infected patients. IL-2 is one of the γ -c chain cytokines, mainly produced by activated CD4⁺ T cells which functions as a T cell growth factor and participates in T cells homeostasis.

We analysed plasma specimens from 18 HIV-1 infected patients treated with ART. Plasma specimens were collected at the start of treatment (defined as 0 time point) and at 2 weeks, 1 month, 3 months, 6 months and 12 months after the start of the treatment. The CD4⁺ T cell number was measured in fresh samples at each time point. All patients included in this study were in an advanced stage of HIV-1 infection and prior to therapy the HIV viral load in the patients was 4.78 ± 0.8 log and the mean CD4⁺ T

cell count was 132 ± 93 cell/ μ l. All patients responded well to ART and at one month from the start of the therapy, the viral load was significantly reduced in all patients to less than 5000 copies/ml, which is an acceptable level of viral suppression as indicated in current WHO guidelines (130). At one year after the start of the therapy the viral load was further reduced to less than 40 copies/ml or undetectable (Fig. 11a). The CD4⁺ T cell number increased gradually after 2 weeks of treatment (192 ± 113 cells/ μ l), 1 month (224 ± 120 cells/ μ l) and one year (391 ± 155 cells/ μ l; $p < 0.001$) (Fig. 11b). Obviously, the CD4⁺ T cells numbers in HIV-1 infected patients were much lower ($P < 0.001$) as compared to CD4⁺ T cell counts in healthy Vietnamese controls.

In general, all cytokines which we measured in the plasma of HIV-1 infected patients were at a level higher than the level found in healthy controls ($p < 0.03$ for IL-7; $p < 0.01$ for IFN- γ ; statistically not significant for IL-2 and IL-1 β), possibly reflecting the dramatic immune activation taking place during in HIV-1 infection. Interestingly, the level of all cytokines increased at 2 weeks after initiation of treatment, with IFN- γ showing the highest concentration (Fig. 11b). This increase in IFN- γ seen at 2 weeks after initiation of ART may reflect an immediate response of the immune system to therapy; the levels of IFN- γ however started to decline again at 1 month after initiation of therapy and the levels stabilized at 3 months at a level lower ($p = 0.03$) than what found prior to therapy. The levels of IL-1 β remained unchanged during 1 year of ART treatment. The levels of IL-7 and IL-2 increased progressively during therapy (Fig 11b) without reaching a statistically significant difference between 0 and 12 months.

The IL-7 levels showed a positive and significant correlation with IL-1 β , IFN- γ and IL-2 (Fig. 12a, b, c). The correlation of IFN- γ and IL-7 may reflect the role of IFN- γ in up-regulation of IL-7 production. The level of IL-1 β also had a positive correlation with IL-7; however, since the patients were in an advance stage of HIV-1 infection only low levels of IL-1 β were detected.

Opposite to previous publications (94, 112, 113), the levels of IL-7 were positively correlated with CD4⁺ T cells although this correlation was not statistically significant (Fig. 12d). This finding can be explained by the observation that after 12 months of ART the CD4⁺ T cells number is low (mean 391) but probably still increasing and IL-7 needs to be maintained at high level in order to continue stimulating T cell reconstitution. In addition, the increase in CD4⁺ T cells during ART may have

beneficial effect on the IL-7 producing cells as indicated in recently published work (46).

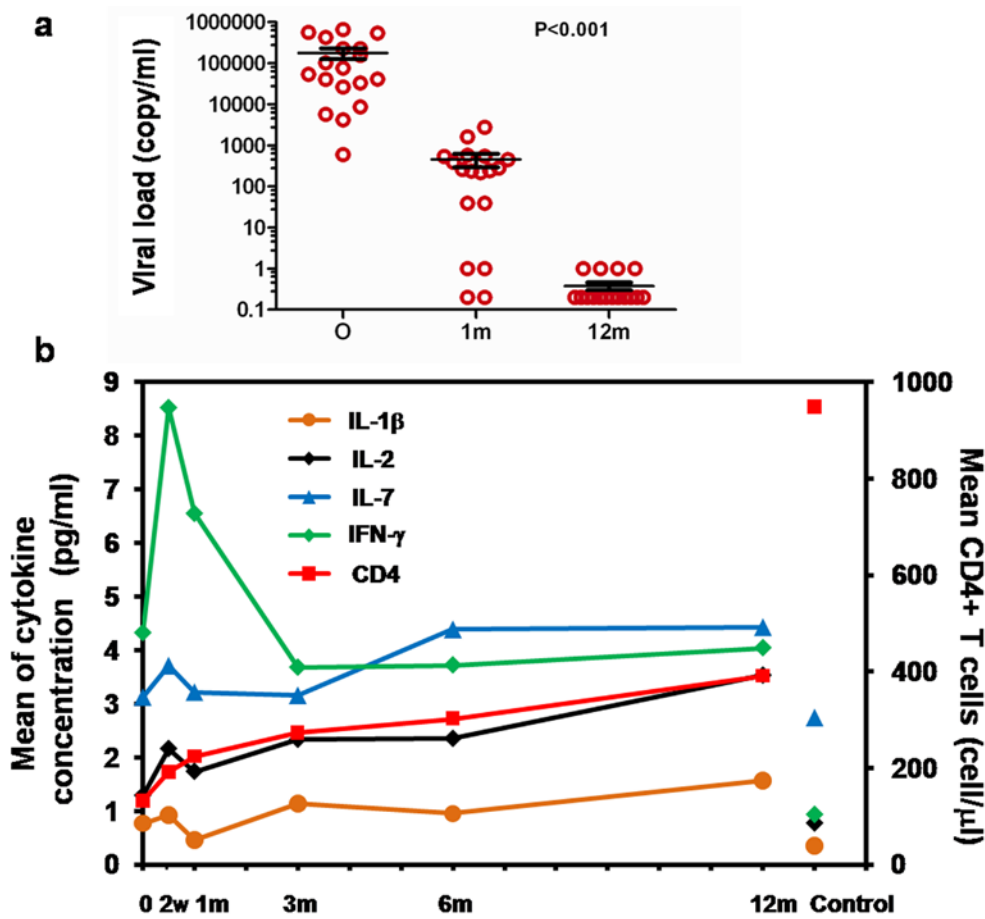


Figure 11. Viral load, CD4+ T cells and level of cytokines in the serum of HIV-1 infected patients receiving ART treatment. (a) HIV-1 viral load (RNA copy/ml) was measured before initiation of ART, then after 1 month and 12 months from the start of therapy; p-value calculated by ANOVA on ranks test. (b) Trends of CD4+ T cell number and cytokines, measured in plasma from 18 HIV-1 infected patients at different time points of treatment, compared to the CD4+ T cell number and level of cytokines measured in 24 HIV negative samples at one time point. The concentration of all cytokine is shown in scales on the left axis whereas the number of CD4+ T cells is shown related to the scale on the right axis, w=week, m=month.

The increased level of cytokines observed in patients at 2 weeks after starting of treatment may be indicating some degree of immune reconstitution inflammation syndrome in the patients (186). The immune system, which was suppressed by HIV-1 infection, is slightly activated when receiving ART and respond to other pathogens.

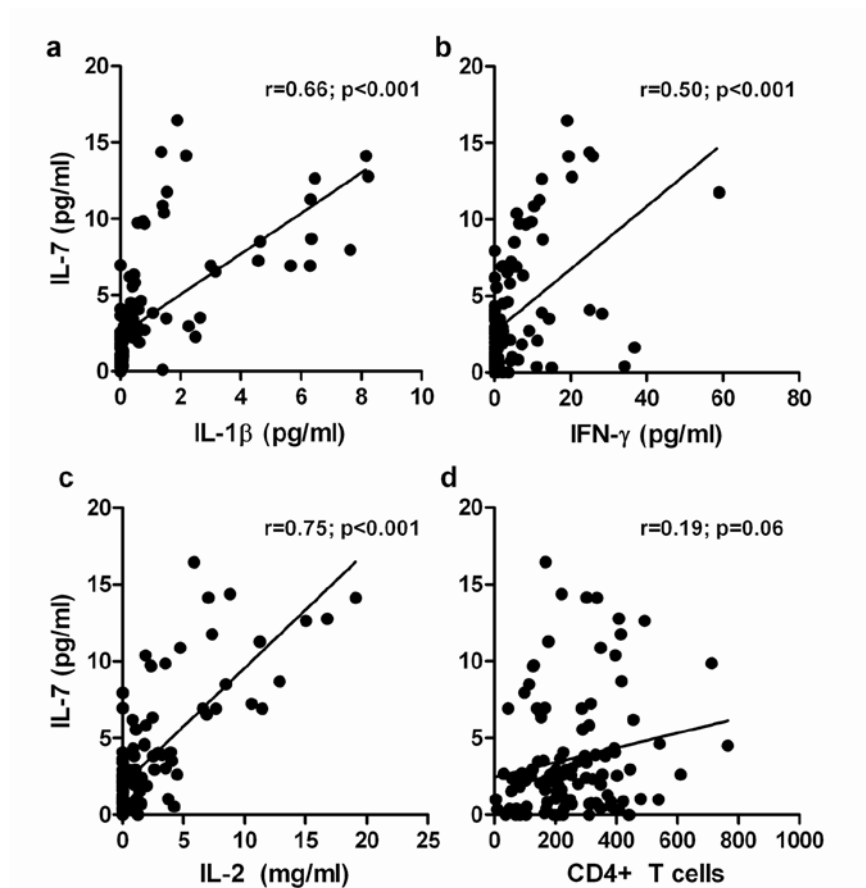


Figure 12. Correlation of IL-7 with IL-1 β , IL-2, IFN- γ and CD4 $^{+}$ T cells in HIV-1 infected patients. For the measurements, 108 plasma specimens obtained at 6 different time points from 18 HIV-1 infected patients were analysed by Luminex assay. The CD4 $^{+}$ T cell number was also determined in the same samples. Correlation of IL-7 with IL-1 β , IFN- γ , IL-2 and CD4 $^{+}$ T cells is shown in (a), (b), (c) and (d) respectively; p -value was generated by Pearson Product-moment Correlation Coefficient test.

The levels of serum IL-1 β have been found to be increased during acute HIV-1 infection; this cytokine is present at very low levels in the sera of HIV-1 patients with chronic HIV-1 infection (165). In addition, high levels of the IL-1R antagonist (IL-1RA), an inhibitor of IL-1 function, were found in plasma during chronic HIV-1 infection and in patients receiving ART (187). It has been shown that the increased IFN- γ levels in plasma are also an indicator for successful ART treatment (187). It is difficult to relate the levels of IL-1 β and IL-7 found in blood with the levels of these cytokines produced in the bone marrow and the gut tissue. The concentration found in the blood may, or may not, be representative of processes taking place in the tissue. Further studies need to be performed to verify if there is any difference in the concentration of IL-7 and IL-1 β in blood, bone marrow and gut tissues of HIV-1 infected patients.

5 GENERAL CONCLUSIONS AND FUTURE PROSPECTIVES

The focus of the present thesis has been on the cytokine IL-7, an important factor for T cell homeostasis. My studies have analyzed 1) the levels of IL-7 in relation to the numbers of different T and B cell populations in specimens from HIV-1 infected patients, 2) the mechanism regulating the production of this cytokine from stromal and intestinal epithelial cells which are important sources to supply IL-7 to T cells and 3) the molecular mechanism leading to up-regulation of CD95 on B cells through factors secreted from IL-7 treated T cells and 4) the relation of IL-7 with the up-regulation of CD95 on B cells.

It was originally described that the levels of serum IL-7 in HIV-1 infected patients changed in relation with the levels of CD4⁺ T cells and that patients presenting with severe lymphopenia had the highest levels of IL-7 in serum (113). Also the results presented in this thesis and results previously presented from our group (94) support the possibility that the IL-7 levels during HIV-1 infection may be related to the number of available CD4⁺ T cells measured in blood. Recent results have however challenged this view indicating that as the results of protracted HIV-1 infection, cells devoted to the production of IL-7 may be damaged and IL-7 production thus impaired (46); if these results will be confirmed by additional studies this may indicate that production of IL-7 may be placed in relation to lymphopenia until the time point when the cells devoted to production of IL-7 are damaged by complex interaction between different cells of the immune system. It would obviously be very important to conduct retrospective longitudinal studies in some HIV-1 infected patients from primary infection to the phase of AIDS to verify whether and in which way the levels of IL-7 in serum fluctuate during HIV-1 infection.

In my work I have found that inflammatory cytokines, including IL-1 β , may suppress the production of IL-7. To understand the connection between inflammatory cytokines and regulation of IL-7 production we have used cell lines in vitro. Elevated levels of IL-1 β , have however been reported together with other inflammatory cytokines (TNF- α , IL-12) in the gut and lymph nodes of HIV-1 patients presenting with acute HIV-1 infection(171) and it is therefore possible that the mechanisms which I studied in vitro may be occurring in vivo already at the initial stage of HIV-1 infection.

I also participated in a study indicating that IL-7, through induction of IFN- γ from T cells, may induce up-regulation of CD95 on B cells and increase the sensibility of B cells for CD95 mediated apoptosis. This is of course a matter of concern in relation to the possibility of using IL-7 as an immunotherapy to promote CD4⁺ T cell recovery and to prevent CD4⁺ T cell depletion during HIV-1 infection. Obviously in vivo the dynamics of apoptosis and proliferation of immune cells may be very complex and not always corresponding to the limited experimental settings which can be established in vitro. The ongoing trials with IL-7 in HIV-1 infected patients will reveal to which extent CD95 upregulation and an increase in CD95 mediated apoptosis occurs on T and B cells as the result of a cascade of events triggered by IL-7 administration.

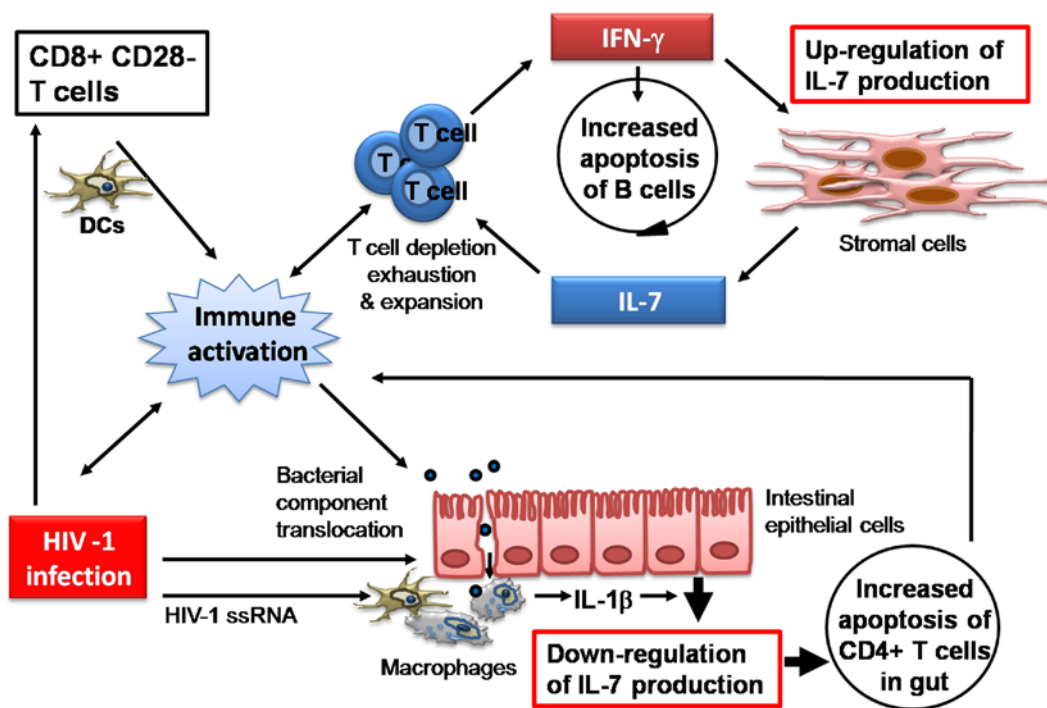


Figure 13. Summary of study findings on the regulation of IL-7 production and role of IL-7 in immune dysfunction during HIV-1 infection.

The results of my studies on the regulation of IL-7 production and the role of IL-7 in immune regulation during HIV-1 infection are depicted in Fig. 13. The content of the figure shows that HIV-1 infection leads to an increase in the CD8+CD28- T cells population. This T cell subgroup which accumulates during HIV-1 infection may contribute to inflammatory reactions and immune activation (paper II). Immune activation and HIV-1 infection lead to a possible increase in bacterial translocation in

the gut which stimulates macrophage in gut tissue to produce high level of IL-1 β . In addition, HIV-1 ssRNA in viremic patients may also stimulate macrophages and DCs to produce IL-1 β . In turn, IL-1 β may down-regulate IL-7 production in the gut compartment thus leading to increased apoptosis of CD4⁺ T cells (paper III). The inflammatory conditions and CD4⁺ T cell depletion in the gut compartment can also lead to increased immune activation. On the other hand, during HIV-1 associated lymphopenia, high level of IL-7 (paper I) and immune activation may induce production of increased level of IFN- γ from T cells, which, in turn, leads to CD95-mediated apoptosis of B cells (paper IV). Also high levels of IFN- γ can stimulate stromal cells to produce increased IL-7 levels (as show in paper III), thus starting a new cycle of B cell apoptosis and also increased immune activation.

I hope to develop some of the concepts which are part of my PhD thesis in my future research work and here I present some initial ideas on my future projects. The majority of observations on the relation between depletion of CD4⁺ T cells and IL-7 cytokine have been obtained studying patients infected with the HIV-1 subtype B, present in the USA and in Europe. It would be interesting to study whether the infection with subtypes other than the B follows the same trends and results obtained for patients infected with subtype B. From this angle I will have the possibility to assess in Vietnam specimens from patients who are infected with the recombinant form of the HIV-1 virus AE (188, 189). To assess the IL-7 levels in the blood of patients infected with the AE recombinant form in Vietnam in relation to CD4⁺ T cells and other subpopulations of T cells may become important in view of the fact that IL-7 is suggested as a possible immune therapy during HIV-1 infection. Thus the follow up of patients identified early (or late) during HIV-1 infection may provide valuable information to decide whether IL-7 therapy may be introduced in combination with ART in Vietnam.

Also I would like to conduct studies on the expression of the IL-7R in HIV-1 infected patients in Vietnam since several publications have shown that the expression of this receptor which is important for T cells to respond to the beneficial effect of IL-7, either physiologically produced or provided by treatment, is reduced during HIV-1 infection. Nothing is known on this particular aspect of immunopathology of HIV-1 infection in patients carrying different HIV-1 genotypes than B. Limited knowledge is available on whether HIV-1 infection alters the IL-7R intracellular pathway and the effect of ART

on these mechanisms; these studies could be conducted in Vietnam through collaboration with the Institute of Biotechnology.

Another aspect which would be interesting to study in Vietnam is the impact of drug use on immunology and immunopathology during HIV-1 infection; the cohorts of patients studied in Sweden are mostly homosexual men and also individuals who acquired their infection through heterosexual contact. It is possible that the route of HIV-1 transmission may impact on the dramatic disruption of gut tissue and dysregulation of production of cytokines pivotal for T cell homeostasis, including IL-7.

The correlation between IL-7 and IL-1 β was investigated in my studies in serum specimens. This may not be reflecting processes ongoing in the affected sites for HIV-1 replication and damage during HIV-1 infection which are the lymph nodes and the gut. I would like to investigate the production of IL-7 and IL-1 β in the gut tissue and I will assess the possibility of obtaining biopsies, in parallel to blood specimens, from the gut of HIV-1 infected patients in Vietnam. This will give information on whether a relation exists on the concentration of IL-7 and IL-1 β in the affected tissue. Important new data have been recently published on the disruption of the network of supporting fibroblasts within the lymph nodes which indicate that IL-7 production from fibroblasts (and possibly stromal cells) may be impaired in advanced phases of HIV-1 infection as a result of immune activation (46). It is obviously impossible to follow the immunological damage occurring in the gut and lymph nodes during HIV-1 infection in man in a time dependent manner because this would request multiple biopsies to be taken from the patients. It is thus very important to utilize biomarkers to follow the immunopathogenesis of HIV-1 infection taking place in gut. A plan should be developed to identify the biological fluid in which to measure biomarkers of events taking place in the gut. This would allow time dependent measurements of cytokines important for the cell homeostasis in the gut.

Specific projects which I would like to continue in collaboration with Sweden are to study how the inflammatory cytokines which I used to modulate the expression of IL-7 in stromal cells affect the survival of plasma cells mediated by stromal cells. Also I am interested in pursuing more measurements of blood cytokines with special focus on IL-1 β and IL-1R antagonists to further understand whether IL-1 β antagonist regulate the impact of IL-1 in immunopathogenesis of HIV-1 infection.

My goal after returning to Vietnam is to contribute to the integration of knowledge within the field of HIV viral diagnostic with the new knowledge in immunology which I acquired during my PhD training in Sweden; among the realistic goals for my near working future in Vietnam is to contribute to improved determination of CD4⁺ T cells and other sub-populations of immune cells. Also I can contribute with teaching in the field of immunology for physician working in the clinical management of HIV infection.

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