Thesis for doctoral degree (Ph.D.) 2010

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Published by Karolinska Institutet.

ISBN 978-91-7409-913-3

Printed by
REPROPRINT AB
Stockholm 2010
www.reproprint.se
Gårdsvägen 4, 169 70 Solna

"Det finns mitt i skogen en oväntad glänta
som bara kan hittas av den som gått vilse"
Tomas Tranströmer

ABSTRACT

The use of combination antiretroviral therapy (ART) has resulted in a substantial reduction in viremia, a rebound of CD4+ T-cells and increased survival for *human immunodeficiency virus type 1* (HIV-1) infected individuals. Unfortunately, ART does not clear the infection or cure the individual from HIV-1. This is due to some unique characteristics of the virus, such as an ability to cause a latent infection in a subset of CD4+ T-cells, and a remarkable genetic variability. This allows HIV to become resistant to treatment under suboptimal conditions (i.e. adherence), and to escape the immune system..
The mechanisms of latency needs to be better understood in order to achieve eradication of HIV-1 infection, and the general aim of this thesis was to study different aspects on HIV-1 latency, with a main focus on the circulating pool of HIV-1 infected resting memory $CD4^+$ T-cells.

In paper I we set out to analyze whether resting memory $CD4^+$ T-cells could serve as a reservoir of founder or resistant viral strains in patients with or without optimal suppressed viremia. Our results indicated a turn-over and replacement of the cell-pool in untreated and suboptimally treated patients. The memory $CD4^+$ T-cell-pool forms an archive of the viral population, but former resistant viral variants are not always found in these circulating cells.

In paper II we wanted to investigate if treatment with high dose intraveneous immunoglobulin (IVIG) in combination with ART could reduce the pool of latently infected resting memory $CD4^+$ T-cells *in vivo*. Our data suggested a reduction by an average of 68% of the HIV infected latent cell pool. The findings from this proof-of-concept study suggest that the reservoir became accessible by IVIG treatment through activation of HIV-1 in the latently-infected resting CD4+ T-cells. IVIG will be further evaluated as an adjuvant to effective ART.

In paper III, we investigated the impact of DNA methylation inhibitors and NFkB activators on HIV-1 latency in resting memory $CD4^+$ T-cells. Cytosine methylation may be an important component that controls HIV-1 latency. We found that a combination of the DNA methylation inhibitor Aza-CdR and the NFkB activator prostratin can reactivate latent HIV-1 *in vitro*. Our data indicate that clearance of HIV-1 from infected persons undergoing antiretroviral therapy may be enhanced by inclusion of these inhibitors.

In paper IV we wanted to investigate to what extent high mobility group box protein 1, HMGB1, alone or in complex with TLR ligands activate latent HIV-1 *in vitro*. HMGB1 is an abundant intracellular protein found in all cells in the body. It contributes to immune activation and has been suggested to have an important impact on HIV-1 pathogenesis. We found that recombinant HMGB1, or HMGB1 as part of necrotic extract , in complex with TLR stimulating ligands LPS, CpG ODN and flagellin, increased HIV-1 replication *in vitro,* as compared to HMGB1 or ligands alone, in a model of latent HIV-1 infection.

Our *in vivo* data suggest that a turn-over of the latent HIV-1 reservoir in resting memory CD4+ T-cells may appear in the natural course of HIV-1 infection in untreated and in suboptimally treated subjects. It is also possible to induce a decrease of the viral reservoirs by additional treatment, in our case IVIg, in patients with controlled viremia.
Our *in vitro* data point at new possibilities to activate latent HIV-1 and further exploration of these mechanisms should be considered.

LIST OF PUBLICATIONS

I. Karlsson AC, Lindkvist A, Lindbäck S, Gaines H, Sönnerborg A.
Recent origin of human immunodeficiency virus type 1 variants in resting CD4+ T lymphocytes in untreated and suboptimally treated subjects.
J Infect Dis. 2001 Dec 1;184(11):1392-401. Epub 2001 Nov 13

II. Lindkvist A, Edén A, Norström MM, Gonzalez VD, Nilsson S, Svennerholm B, Karlsson AC, Sandberg JK, Sönnerborg A, Gisslén M.
Reduction of the HIV-1 reservoir in resting CD4+ T-lymphocytes by high dosage intravenous immunoglobulin treatment: a proof-of-concept study.
AIDS Res Ther. 2009 Jul 1;6:15

III. Kauder SE, Bosque A, Lindqvist A, Planelles V, Verdin E.
Epigenetic regulation of HIV-1 latency by cytosine methylation.
PLoS Pathog. 2009 Jun;5(6):e1000495. Epub 2009 Jun 26

IV. Lindkvist A, Nowak P, Troseid M, Abdurahman S, Sönnerborg A
HMGB1 and Toll like receptor ligands synergistically induce HIV-1 replication in vitro
Manuscript 2010

CONTENTS

LIST OF ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
CD	cluster of determination
CXCR	CXC chemokine receptor
DNA	deoxyribonucleic acid
env	envelope gene
gp	glycoprotein
HAART	highly active anti-retroviral therapy
HIV	human immunodeficiency virus
HMGB1	high mobility group box protein 1
IL	interleukin
LAV	lymphadenopathy associated virus
LTR	long terminal repeat
mRNA	messenger RNA
nef	negative regulatory factor gene
NFAT	nuclear factor of activated T-cells
NF-kB	nuclear factor kappa B
NNRTI	non nucleoside analogue reverse transcriptase inhibitor
NRTI	nucleoside analogue reverse transcriptase inhibitor
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PI	protease inhibitor
pol	polymerase gene
P-TEFb	positive transcriptionelongation factor b
RAGE	receptor for advanced glycation endproducts
rev	regulator of virion gene
RNA	ribonucleic acid
RT	reverse transcriptase
TAR	tat-responsive element
tat	trans-activator gene
TLR	toll-like receptor
UNAIDS	joint united nations program on HIV/AIDS
WHO	world health organization

vif	virion infectivity factor
vpr	viral protein Rr
vpu	viral protein

Introduction

The world first became aware of AIDS in the summer of 1981 when a series of opportunistic infections seemed to affect only previously healthy homosexual men in New York and San Francisco [1-3]. Two years later it was discovered that these infections was caused by a retrovirus, and that target groups were not limited to homosexual men, indeed it was a disease that could affect us all .More than 25 years have passed since then and no other virus have been examined so thouroughly as HIV. Tremendeous efforts have been made in order to reveal the etiology, molecular virology, epidemiology and pathogenesis of HIV. Discoveries lead to generations of effective therapies and to development of a great variety of tools for diagnostics and prevention.

A search blast on PubMed: generates approximately 210 000 hits on "HIV" and 170 000 "on AIDS. In comparision with hits for some other common viruses such as " herpes" 50 000 "HCV" 27 000 "parotitis" 10 000 "adeno" 5000 "rhino" 86 000 and"influenza" 54 000 , this mirrors the enormous achievements that have been made in HIV research during the years And yet, the struggle to control the infection is far from over.

Discovery

Human immunodeficiency virus type 1 (HIV-1) was first isolated and identified by French researchers Barré-Sinoussi and collegues in 1993 and was temporarily named LAV (lymphadenopathy-associated virus) [4]. The following year, American researchers isolated an identical virus and named it HTLV-III (human lymphotropic virus type III) [5, 6]. Soon after identification of the virus the complete genome was sequenced and the viral proteins could be characterized [7]. In 1986 the virus received its final name, human immunodeficiency virus (HIV). Within that year yet another virus was isolated from West African patients with AIDS like disease [8].This virus was similar but not identical to HIV and subsequently the viruses got the names HIV-1 and HIV-2. Both viruses cause AIDS and share the same transmission patterns, although there are some important differences, infection with HIV-1 is much more common and HIV-2 is less pathogenic than HIV-1 [9, 10].

The oldest verified case of HIV infection dates back to 1959 and comes from a frozen blood sample found at a STD clinic in Congo Kinshasa. [11]. However, there is also one suspected case dating back to 1934 , but it has not been verified due to lack of tissue or blood samples.

The French researchers Luc Montagnier and Barré Sinoussi received the Nobel prize in 2008 for their discovery.

Origin

The key to understanding the origin of HIV came with the discovery of closely related viruses in a wide variety of African primates, the simian immunodeficiency viruses (SIVs) [12].These viruses have been isolated from several primates and so far there is no evidence that SIVs cause disease in its natural hosts.
Phylogenetic analysis show that there have been several cross-species transmissions to humans. HIV-2 is supposed to originate from the sooty mangabe monkeys, whereas three jumps from chimpanzees and one from gorilla to humans can explain the four groups among HIV-1; the M (major), O (Outlier) and the N (non M non-O)groups [12, 13] and the recently proposed P group [14].
Within the M group there are currently 11 genetically distinct subtypes A-K which have a a distinct geographic distribution worldwide, although there is a rapid spread of different subtypes to new areas of the world. Occasionally, two viruses of different subtypes "meet" in the cell of an infected person and the genetic material from these viruses combine to create a hybrid virus. If this "new" strain survives long enough to infect at least two separate indivuals, it is referred to as "circulating recombinant forms", CRFs.
HIV-2 is divided into eight groups, A-H, and each group of HIV-2 represents at least one separate sooty mangabey to human transmission event [15, 16].

Pandemic

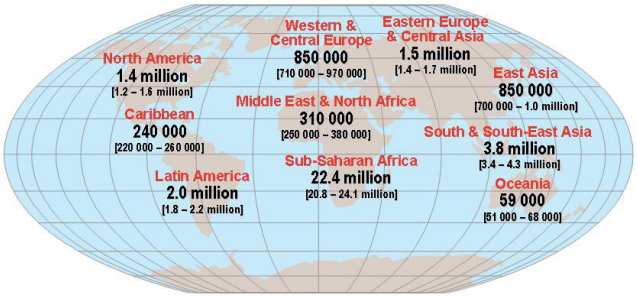
HIV-1 has spread and are still spreading in successive waves in various regions around the globe. Today, it is estimated that approximately 33 million people are infected with HIV and 25 million people have died due to HIV/AIDS [17].
The majority of the cases are found in Sub-Saharan Africa with the numbers of infected individuals unfortunately increasing in the region, and the situation is similar in Asia. However in Latin America and in the Western world the numbers of newly infected individuals seem to be stabilizing.

The HIV prevalence is low in Sweden. Until December 2009, approximately 8900 HIV infected individuals have been reported to the Swedish institute for infectious diseases, and 5000 of those are still living with the infection [18].

World Health Organization

UNAIDS

Adults and children estimated to be living with HIV, 2008



Total: 33.4 million (31.1 – 35.8 million)

HIV-1 virology

The mature HIV has the phenotypic characteristics of a Lentivirus of the Retroviridae family, with a cone-shaped core composed of the viral p24 Gag capsid (CA) protein [19] surrounded by a matrix (MA) that lines the inner surface of the viral envelope. The virion has a spherical heterogeneous morphological shape with a diameter of approximately 100 to 120 nm [20]. On the inner side of the viral core structure , thightly bound to the nucelocapsid, two copies of single stranded viral RNA are contained, as well as the viral proteins reverse transcsriptase (RT), protease (PR) and integrase (IN).

The outer layer of the virus, the envelope, is derived from the host lipid bilayer with host cell proteins and anchored viral glycoproteins (gp), surface gp120 and transmembrane gp41. These glycoproteins comes as trimers on the surface and are of major importance for the binding of the virus to the CD4 molecules on HIV-1 target cells.

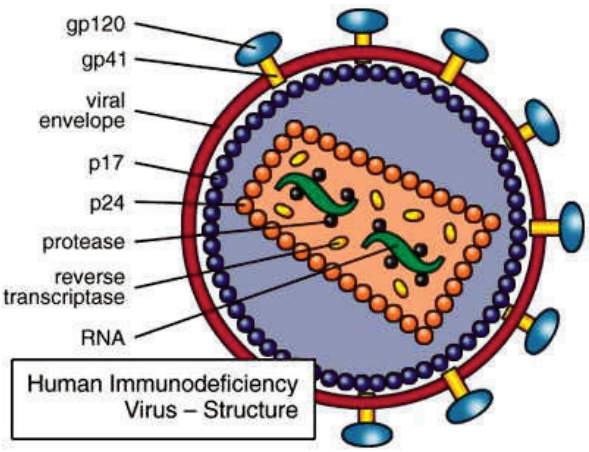


Figure 1. The mature HIV-1 virion

Genomic structure

The HIV-1 genome consists of two positive sense, single-stranded RNA molecules of about 9.2 kilobases (kb) each, with nine open reading frames.

Like all other retroviruses, HIV-1 provirus contains the structural genes *gag*, *env* and *pol* encoded by three open reading frames. The *gag* gene codes for the viral core proteins (MA;p17,

CA;p24, NC;p7 and p6), the *env* gene for the surface envelope glycoproteins (p120 and p41) and the *pol* gene for proteins required for viral replication reverse transcriptase (RT;p66/51), protease (PRp11) and integrase (IN;p32). Other HIV-1 gene products, from the remaining six open reading frames, are a variety of viral regulatory and accessory proteins (*tat, rev, nef, vpr, vpu* and *vif*) that are essential for viral replication and infection [21, 22].

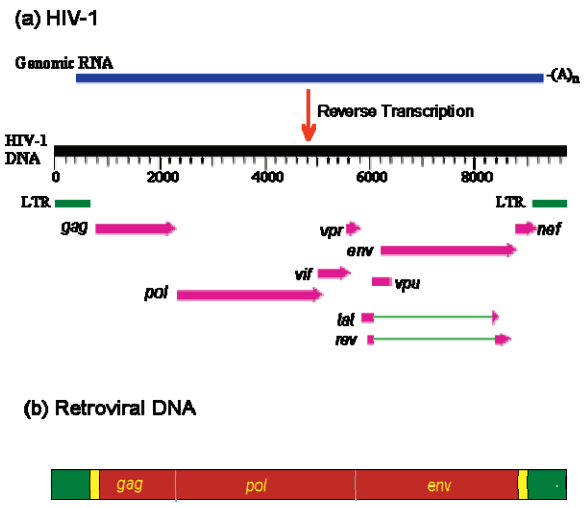


Figure 2. a) The genome organization of HIV-1 (b) General organization of retroviral DNA Adapted from

Replication and transcription

All retroviruses are viruses that replicate via DNA intermediates using the viral enzyme reverse transcriptase. HIV-1 replication is initiated through an interaction of the viral gp120 and the CD4 receptor on the target cells; T-lymphocytes, macrophages, monocytes, dendritic cells and microglial cells [23-25]. The binding induces a conformational change of the gp120 which exposes the fusion protein gp41 and binding sites for co-receptors, mainly the CCR5 and CXCR4 from the chemokine receptor family [26]. This interaction results in partial uncoating of HIV-1 core particles and the RT synthesizes a complementary DNA strand using the single stranded viral RNA genome as a template. Through RT ribonuclease activity, the DNA strand is removed from the RNA strand and a second DNA strand is synthesized. The dsDNA is then transported from the cytoplasm into the nuleus of the cell whereafter it is ligated into the host chromosomal DNA by the viral enzyme integrase and becomes the proviral DNA .

The integrated provirus are flanked by two long terminal repeats (LTR) comprised of three regions, U3,R and U5. The LTR located at the 5´end contains enhancers , promoters and binding sites for cellular and viral transcription factors, elements that are crucial for HIV-

transcription [27, 28]. The viral transcription is to a great extent regulated by the viral Rev and Tat proteins and other cellular transcription factors induced during cell activation [29] (This will be discussed later in the Thesis)

Activation of the provirus initiates transcription of the full-length structural viral RNA and concomitantly spliced mRNA are produced. The differential expression of the mRNAs is controlled by the Rev protein. When Rev reaches a certain level in the cytoplasm export of unspliced and incompletely spliced viral RNAs from the nucleus to the cytoplasm is enabled and expression of structural proteins is activated. Provirus that lack the Rev activity are transcriptionally active but does not produce virions.

The viral proteins assemble at the host cell surface where the Env protein, necessary for buddingthrough the host cell membrane, are inserted. Shortly after budding, the gag and gag-pol precursors are cleaved by viral protease and new infectious virions are produced[22, 30-32].

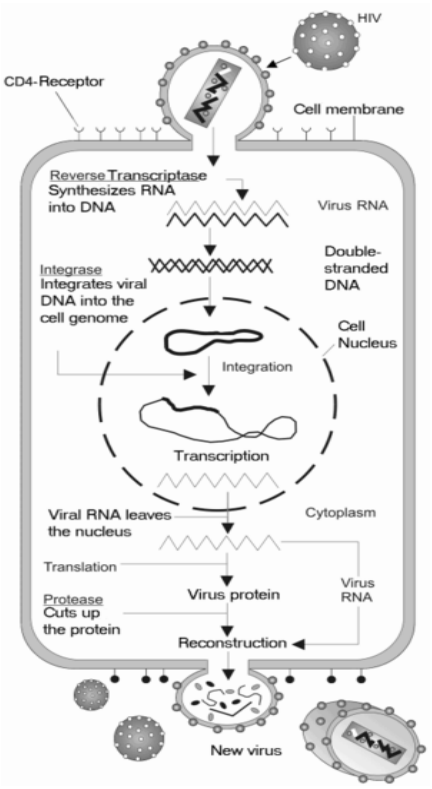


Fig3. The HIV-1 replication cycle

The natural course and consequences of HIV-1 infection

The virus is transmitted via unprotected sexual contacts, exposure to HIV contaminated blood and blood products and vertically from mother to child. HIV-1 is present as free virus particles and as virus within infected cells and has been isolated from blood, seminal fluid, pre-ejaculate, vaginal secretions, cerebrospinal fluid, saliva, tears and breast milk [33-36]. However, the concentrations from saliva and tears are extremely low [37].

For sexual transmission, HIV usually gains entry through mucosal surfaces (vaginal/rectal) where it infects dendritic cells and CD4+ T-cells in the underlying submucosa [38]. The infected cells migrate to regional lymphnodes where viral replication takes place and T-cell responses are initiated [39, 40].
Among injecting drug users the virus is usually transmitted through shared HIV contaminated needles, although sexual transmission also occurs within this category.

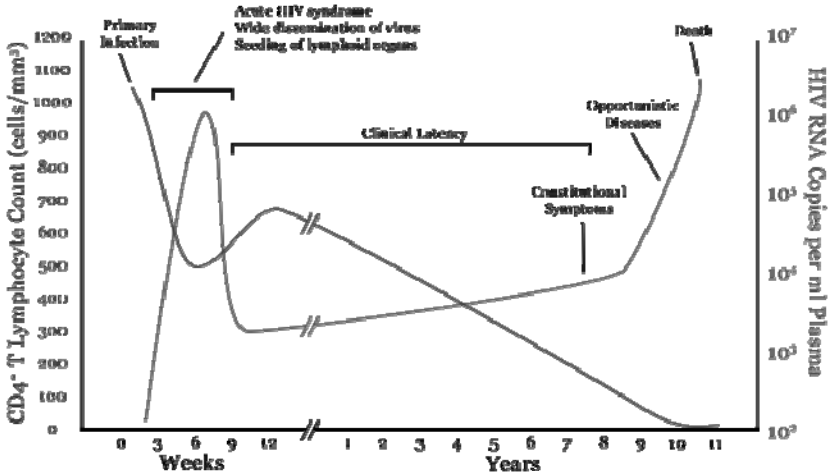


Fig 5.The different phases of HIV-1 infection

One to three weeks after infection a primary HIV- infection (PHI) occurs [41]. This can be either symptomatic or asymptomatic. The symptoms, and also the degree of the symptoms, differ between individuals but include rashes, myalgia, sore.throat, high fever, gastrointestinal complaints, CNS disorders and lymphadenopathy [42-44]. After the primary infection the viral load drops to a steady level and a period of clinically asymptomatic latency follows. This clinical latency can last for several years, in mean ten years without antiretroviral therapy.

However, an accelerated loss of CD4+ T-cells and an increase in viral load lead to dysfunction of the immune system and a susceptibility to AIDS-associated opportunistic infections and tumors [45, 46].
The development of AIDS will be dependent on factors such as transmission route, initial viremia, symptomatic or asymptomatic PHI, viral phenotype, gender and age [47-49].

Immune activation

Chronic immune activtion is a hallmark of progressive HIV infection and one of the strongest predictors of disease progression, indeed it better predicts disease outcome than plasma viral load [50-53]. Immune activation is a crucial factor that distinguishes pathogenic SIV infection from non pathogenic SIV infection in nonhuman primates [54, 55]. SIV infection in its natural hosts is associated with low immune activation despite high viral load. Manifestations of chronic immune activation include polyclonal B cell activation [56], increased T cell turnover [57] T-cell activation [58], and increased levels of proinflammatory cytokines in serum [59]. The consensus among researchers is that immune activation over time is detrimental to the HIV-1 infected person, although it may have some initial beneficial effects on T-cell proliferation and restoration of tissue memory T-cells [60].

HIV is a virus that generates its own substrate for replication. Immune activation results in the generation of activated CD4+ T-cells further driving viral replication [61, 62]. High turn-over and clonal exhaustion of T-cells may ultimately drain the memory T cell pools [63, 64]. However, the direct effects of the virus on T-cell death does not explain the significant loss of CD4+ T-cell that is seen during disease progression.
Recent studies have provided a direct link between immune activation in chronic HIV infection and the mayhem that occurs to mucosal surfaces after primary infection [65]The damage to the gastrointestinal mucosal surface allows translocation of microbial products that may exacerbate immune activation [66]. Thus, to understand the pathogenesis of progressive HIV infection, it is of utmost importance to identify factors that underlies systemic immune activation.

Treatment

During the early days of the HIV pandemic treatment was given as mono-and/or dual therapy. This turned out to be a supotimal strategy that soon resulted in a high degree of resistance to the drugs [67-69]. The clinical benefits were not satisfactory and a more potent strategy was urgently needed. In 1996 a combination treatment was initiated, the “highly active antiretroviral therapy” (HAART), and with great success. This approach has dramatically decreased the

numbers of deaths and highly increased the quality of life in HIV-1 infected individuals [70]. It was initially believed that effective treatment early during the primary infection could be a way of conquer the HIV-1 infection and enable complete eradication of the virus. However, HIV-1 is still present in the lymph nodes and replication competent HIV-1 can be recovered from latently infected memory T-cells in blood [71, 72]. Furthermore, drug resistant viruses that develops in the setting of incompletely suppressive therapy can be deposited in the latently reservoir and thereby permanently limiting treatment options [73, 74].
Today, five different classes are used in combinations of at least three drugs. These classes are:

- Nucleoside analog reverse transcriotate inhibitors -NRTI
- Non- nucleoside analog reverse transcriptase inhibitors - NNRTI
- Protease inhibitors PI
- Integrase inhibitors
- Entry inhibitors

Viral evolution and drug resistance

The mutation rate is very high in HIV-1 due to lack of proof-reading activity of the RT [75, 76]. This results in a remarkable genomic heterogeneity [77] and a low genetic barrier towards resistance development. [78-81]. Other sources of the variability is the host RNA polymerase II and recombination events .

HIV-1 resistance can be defined as an altered phenotype resulting from a change in viral genotype that improves the viral replication in the presence of an inhibitor. Most of the new HIV variants have little or no impact on viral function or fitness and many will be lethal to the virus. However, some mutations may change the replicative capacity of the virus conferring resistance to one or more antiretroviral drugs. The most common mutations are the substitution mutations of one nucleotide for another. Deletions and insertions have also been observed but not to the same extent

The nucleotide substitutions are either synonymous and do not change the amino acid, or non-synonymous that change the amino acid. The genomic regions are not equally affected by substitutions and the env region is the most variable region [82, 83]. Env is under a high immunological pressure and variation helps the virus to escape the immune system. The pol region, on the other hand, is less error prone since the virus need to maintain its ability to replicate.

Antiretroviral drug treatment changes the selective pressure on the viral population. Some viral variants may display natural resistance to a certain extent and these variants will continue to replicate in the presence of an antiviral drug which increases their population in comparision to the wild-type. These escape mutations can accumulate more mutations that can be beneficial or detrimental to the virus. Eventually, an outgrowth of a fit resistant stain might result in drug failure.

All the antiretroviral drugs will select for certain mutations associated with drug failure and outgrowth of resistant virus. The virus may also display cross-resistance to other drugs, which means that resistance to a drug can be conferred by mutations selected by another drug. There is no cross-resistance between different classes of antiretroviral drugs, it arises to drugs within a given class. However, it is possible for RT to carry both NRTI and NNRTI-associated mutations and therefore be resistant to inhibition by both drugs.

HIV-1 latency

Viral latency is a characteristic of many viruses, including retroviruses adenoviruses and many members of the herpesviridae, although the nature of latency differs between them. The virus may exist in a truly latent noninfectious form, possibly as an integrated genome or an episomal agent, or as an infectious and continuously replicating agent, termed a persistent viral infection.

HIV-1 do not have a "true" latent state between episodes of active viral replication. Instead, HIV-1 latency is mainly a consequence of the virus´s preference for infecting activated CD4+ T-cells that have the ability to revert to a resting state after activation [84-87].

Viral dynamics and HIV-1 latency

Potential reservoirs for HIV-1 are for example; follicular dendritic cells which are not infected but can trap the virion on its surface, macrophages/monocytes, CNS, the male genital tract and the thymus [71, 88-90]. However, the most important reservoir, and the most significant obstacle to eradication is thought to be the resting memory CD4 + T-cells. This reservoir is established very early during primary infection and has a half-life estimated to more than 60 years [91-93].

HIV-1 replicates very fast in infected individuals. Each day, the number of newly created viral particles has been estimated to be 10^{10}, and the virion half life in plasma is less than 6 hours [94, 95]. The majority of the replication takes place in productively infected activated CD4+ T-cells in the peripheral blood and in lymphoid tissues.
Analysis of the kinetics of viral decay in plasma following initiation of ART to therapy naïve individuals has revealed a viral decay in at least three distinct phases.
The first phase of decay occurs within two weeks after initiation of therapy. The viral load drops by two log and represents the clearance of free virus in plasma and the loss of CD4+ T-cells that were infected before initiation of treatment [95, 96].
After the initial drop, plasma virus declines more slowly which probably reflects infected macrophages that are less susceptible to the cytopathic effect of the virus and follicular dendritic cells that comprise a large fraction of the viral burden in an infected patient [97-99].
After several months on effective antiretroviral thearpy, the viral load in plasma is reduced to clinically undetectable levels in many patients [71, 94, 100]. This is referred to as the third phase of decay [101]. Despite clinically undetectable viral load, a low level of virus is still

present in plasma and in other compartments. This residual viremia can only be detected with very sensitive methods [102, 103].

Two general forms of HIV-1 latency have been detected and depends on whether the viral genome has integrated into the host genome or not. These two forms of latency are referred to as pre-integration and post-integration latency. The dominant form of HIV-1 DNA in untreated or viremic subjects is found in preintegration complexes and resides in the cytoplasm as fully or partially transcribed linear DNA or as long terminal repeat (LTR) circles [85, 87, 104]. This is commonly observed following the fusion of HIV-1 to resting, unactivated cells [87]. A small fraction of this unintegrated DNA is replication-competent but labile and will degrade within hours, or at most within a few days, unless the resting T-cells are activated [84, 85, 87, 93, 103, 105]. This labile form of latency may be cleared during the first weeks of effective ART.

Stable integration of the resting memory T-cells, or post-integration latency, is established soon after primary HIV-1 infection and even early initiation of ART is unable to prevent its establishment [73]. The persistence of this pool is due to an extremely long half life of these cells, which is a consequence of the natural course of T-cell memory, and the integrated DNA is suggested to persist until the cell is activated or dead.

The number of resting memory T-cells with an integrated provirus is very low. Roughly, 1 in 10^6 million resting CD4+ T-cells harbor integrated and replication competent provirus, predicting a total body load of $<10^7$ latently infected cells[93, 104].

Furthermore, a persistent low-level viral replication may lead to a replenishment of the resting T-cells and a prolongation of the half-life of the reservoir [93, 103-108].

Mechanism of latency

HIV-1 latency is controlled by the metabolic and activation state of the host cell. The only difference between a latently infected and an uninfected CD4+ T-cell is the presence of the transcriptionally silent provirus. Hence, there re no markers that can distinguish these cells from each other.
It is known that the retroviral DNA has a preference for integration within introns of transcriptionally active genes [109-111].The mechanism behind this is unclear, but the nature of the site has impact on HIV-1 trancription levels in active CD4+ T-cells [112].

The main regulators of viral expression are the viral trans-activator of transcription, Tat, and host cellular transcription factors. Tat binds to the transactive responsive element (TAR) which is a RNA steem loop structure located at the 5´end of all viral transcripts. After binding, it

recruits the cellular transcritption factor b (P-TEFb) that enables elongation of cellular RNA polymerase II and generates full-length RNA transcripts [113, 114, 115]. Absence of cellular activation signals and of viral Tat generates short RNA transripts that will be aborted [113, 116, 117]. Also, absence of cellular transcription factors greatly affects HIV-1 latency. When T-cells are activated, nuclear factor of activated T cell (NF-AT) and NF-kB are recruited to the nucleus, and synergistically with Tat, gene expression is upregulated [28, 118-120]. Active NF-AT and NF-kB are missing in resting T-cells.

The chromatin conformation at the integration site decides whether the provirus is transcriptionally active when the HIV-1 DNA is integrated into the host genome . For high level HIV transcription it is essential that cellular transcription factors can bind to the LTR, and heterochromatin structure may inhibit that interaction [112]. Two exact positioned nucelosomes can be found in the 5`LTR. When one of these (nuc-1) is disrupted, the chromatin is remodeled and activation of HIV-1 gene expression can start [121]. This disruption can be induced by histone deacetylases (HDACs), and are a result of the recruitment of histone deacetylasetransferase by NF-kB or Tat. This mechanism may be used therapeutically in the form of HDAC inhibitors . [122] Other compounds have also been found to have a similar effect on a nuc-1, i.e phorbol esthers and tumor necrosis factor alpha (TNF-a).

Attempts to decrease the latent reservoir

Given the persistent nature of the latent reservoir, it is unlikely that intensification of ART will be enough to eradicate HIV-1. Several strategies for dealing with this have been suggested (see next page). However, a deliberate activation of latently infected cells will be required to purge the long-lived reservoir of latently infected resting CD4+ T-cells.

Treatment	Mechanism	Potential efficacy	Outcome
IL-2 [123-126]	Direct T-cell activation	The pool of replication competent HIV-1 from resting CD4+ T-cells decreased.	Rebounding plasma HIV-1 RNA within weeks after therapy interruption
IL2 and IFN-γ [127]	T-cell and macrophage activation, release of proinflammatory cytokines	Decrease of proviral DNA and positive effects on immune reconstitution	Rebounding plasma HIV-1 RNA levels within weeks of therapy interruption
IL-2 and OKT3 [128-130]	Direct t-cell activation and release of proinflammatory cytokines	Immune activation, T-cell activation and proliferation, stimulation of HIV-1 replication, long-lasting CD4+ T-cell depletion	No significant decrease of total HIV-1 DNA
IL-2 and low-dose OKT3 Hydroxyurea/Didanosine **[131, 132]**	Direct T-cell activation	Undetectable replication competent virus after treatment, plasma viral RNA either undetectable or <5 copies/ml	Viral rebound in plasma within weeks after therapy interruption
Cyclophosphamide [133]	Elimination of latently infected cells and available uninfected target cells	Lower CD4+ and total lymphocyte counts	No reduction of virus in peripheral blood and lymphnodes
Valproic acid Enfuvirtide **[134-136]**	HDAC inhibitor; relaxation of heterochromatin structure resulting in transcriptional activation of provirus	Accelarated clearance of HIV-1 from CD4+T-cells and a shortening of the half-life of reservoir	Viral rebound in plasma within weeks after therapy interruption

Aims

The general aim of the thesis was to view different aspects of HIV-1 latency, both in vitro and in vivo, with main focus on resting CD4+ T-cells as a reservoir in HIV-1 infection.

Specific aims

(I) To analyse whether resting memory CD4+ T-cells could serve as a reservoir of founder and earlier detected resistant viral strains in patients with or without optimal suppressed viremia

(II) To investigate if treatment with high dose intraveneous immunoglobulin (IVIg) could reduce the pool of latently infected resting memory CD4+ T-cells *in vivo* in subjects undergoing effective antiretroviral therapy with suppressed viremia.

(III) To investigate the impact of DNA methylation inhibitors and NFkB activators as a way to reduce the latent HIV-1 latency pool *in vitro*

(IV) To investigate to which extent high mobility group box protein 1(HMGB1) alone or in complex with TLR ligands activate latent HIV-1 *in vitro*.

Material and methods

For detailed information about Material and methods used in this thesis, see respective papers.

Ethical clearance

The studies included in the thesis were performed after approval from the Regional Ethical Committees. All subjects included gave their informed consent prior to study

Statistical analysis

For details about statistical methods and analysis used in the thesis, see respective papers.

Results and Discussion

In this thesis I have focused on different aspects of HIV-1 latency. Since my research group has a long-standing focus on translational research, the articles reports both *in vivo* and *in vitro* experiments of relevance for this research question. As described in the introduction, latency is a consequence of the fact that the virus replicates in activated T-cells, and that these cells have the ability to return to a resting state and persist as memory cells, thereby harbouring integrated provirus. Subsequently, viral latency represents a significant barrier to curing HIV-1 infection and it is therefore crucial to understand.the molecular mechanisms that are involved. Hopefully my research will bring new insights and ideas on how to purge this reservoir of latent virus.

Paper I

Study background

Latently infected resting memory CD4+ T-cells have been well characterized in patients on effective antiretroviral treatment, and it is clear that this reservoir prevents eradication of the virus despite a successful treatment regimen.
At time of initiation of ours study, only a few studies had been published on the topic. Especially, the knowledge of the viral kinetics in untreated individuals and in patients that have received suboptimal therapy was limited. We therefore set out to characterize the viral populations from such patients.

To visualize the divergence and diversity of the viral population there are several methods to choose from. In this study we used direct sequencing and cloning. Direct sequencing is an excellent tool for analysis of the major and minor sequences of the viral population in a sample, but does not give you information about individual sequences [93, 103-108]. Also, the detection limit of minor quasispecies is high, 15-30%. However, with this population-based approach we could rapidly retrieve information of sequences from a larger number of blood samples drawn at numerous time-points. By the time we performed the study there were mainly two different techniques available to allow studies of individual sequences (quasispecies), i.e limiting titration and cloning. Limiting titration is expensive and time-consuming and for our purpose, with the large amount of sequences we aimed at, cloning seemed like the most realistic choice. We used a high number of input templates to confirm a high number of unique clones and a lower probability of resampling errors [137, 138]. Also to avoid Taq-introduced errors, a high fidelity enzyme was used.

Rapid turn-over and replenishment of HIV-1 in peripheral blood cells may occur in individuals with detectable viremia

Plasma and resting memory CD4+ T-cells from peripheral blood cells were obtained from three patients with primary infection (PHI) in order to analyze virus from these compartments. Two of the patients were untreated and one had received treatment from the first week after onset of symptoms. We also analyzed virus from the same compartments from five therapy experienced patients who previously had developed resistance mutations.
Phylogenetic analysis was performed on sequences from the two untreated PHI patients and from the treated PHI patient. Sequences from the two untreated subjects showed that the viral population from the resting memory T-cells and the corresponding plasma population formed a branch that were separated from the initial plasma population, but also that virus from the corresponding compartments were distinct from each other. When we compared sequences from the treated PHI patient we found almost identical sequences from the plasma and the resting T-cells.

We went further by analyzing virus from patients that had developed resistance mutations due to suboptimal therapy. In four out of five patients, we detected primary PI mutations in plasma virus during virological failure and also primary and /or secondary mutations associated with RTI resistance were found in all of the five patients. The primary PI mutations disappeared in three of the patients when the regimen was changed towards a non-PI containing regimen. For the RTI associated mutations a different pattern appeared; some mutations were not detectable after therapy interruption but others persisted for several years, probably a consequence of introduction of new drugs and possible cross-resistance.
As for the resting CD4+ T-cells from these patients, most of the mutations related to NRTI and NNRTI, as well as for the primary PI-resistance associated mutations, could not be found.. However, when comparing secondary PI mutations from the two compartments, two patterns emerged. Some of the mutations were found in all of the analyzed clones while others could not be detected among virus from the resting CD4+ T-cells. Är detta i den sista meningen viktigt??

In three patients we were able to obtain resistance mutations patterns from plasma and resting CD4+ T-cells at corresponding time points. The mutations in the two compartments were similar in two of the patients, while we found a pronounced difference in the third patient.

A latent reservoir of HIV-1 is established very early during HIV-1 infection and protects the virus from therapeutic intervention [72, 91, 92, 101]. Due to our findings, the initial interpretation was that there is a turn-over of this cell pool in untreated and sub-optimally treated patients in peripheral blood. The consequence of this would be that the latently infected memory T-cells in the periphery represent a more short-lived major reservoir for archived

founder virus. However, our initial conclusion might be questionable due to the probability of the existence of pre-integrated virus in viremic patients. The half-life of pre-integration latency is approximately one day, and a few days of culture are required for pre-integration forms of latent virus to decay [87, 139, 140]. In our patients the findings could thus rather reflect recent circulating viral populations close to the time point when the different compartments were analyzed. Therefore, the proportion of integrated archived founder sequences might "drown" in comparison with the pre-integrated complexes that would infect the resting cells due to low grade viremia and dominate the viral population upon cellular activation. However, it should also be noted that the handling of our samples from venepuncture to analysis in vitro took in median 30 hours (eller vad det nu var) so a substantial decrease of pre-integrated HIV DNA could have had happened and therefore our results may not necessarily represent pre-integrated virus.
These circumstances were taken under consideration in the following projects.

Paper II

Study background

The idea for this proof of concept study originates from a case study on a HIV-1 infected patient with Guillan-Barré syndrome [141]. He had been treated for HIV-1 as well as for his autoimmune condition with ART and intraveneous immunoglobulin (IVIg) respectively. During the IVIg treatment a viral blip was detected, but the VL soon returned to baseline levels. After termination of ART it took several months before HIV RNA became detectable. This raised the hypothesis that IVIg contributed to activation and release of archived virus from latently infected CD4+ T-cells, and thereby possibly decreased the size of this cell pool. If so, this could explain the relatively long period of undetectable viral load after ART interruption.

The pool of latently infected resting CD4+ T-cells is reduced in patients after IVIg treatment

To test this hypothesis, nine patients who had been on effective ART for at least two years and with undetectable VL for approximately 1.5 years, were enrolled. They received 30g of IVIg per day for five consecutive days. HIV RNA was quantified on several occasions, including the five days as IVIg was given, and the pool of resting memory CD4+ T-cells was quantified at baseline and 8 or 12 weeks after IVIG treatment.
In seven out of nine patients we were able to quantify virus from the resting cells and in five of these positive patients we detected a decrease of the latent pool. Plasma HIV RNA was

detectable in these five patients two weeks after initiation of IVIg treatment, and the highest plasma viral load correlated well with the levels of IUPM (infectious units per million cells) quantified before IVIg treatment. These patients had detectable plasma HIV-1 RNA at baseline as well, but the levels went below the detection limit at the time for follow-up.

We went further by comparing viral sequences from plasma and supernatants from activated T-cells using single-genome-sequencing (SGS) in two patients. We found a close relationship between virus from plasma and the T-cell reservoir, and the sequences clustered together nicely in a distinct branch in the phylogenetic trees. We presume that the sequences that we obtained from the activated T-cells represented an oligoclonal expansion in the culture of the most replication-competent HIV-1 in the resting T-cell population.
The sequence data together with the results from the quantification assay indicate that HIV in plasma originated from the pool of latently infected T-cells.

We postulated that the described effect by IVIg was mediated through cytokines since it is unlikely that IVIg itself could activate the cells directly. Therefore, we analyzed cytokines IL-2 and IL-7, A pattern was found among the patients that had a reduction of the pool size of resting cells. During the first eight days after IVIg treatment the levels of IL-7 increased while the levels of IL-2 remained unchanged. Both of these two cytokines have been used in attempts trying to reduce the pool of latently infected T-cells.

We have also investigated the differences in activation markers before and after IVIg treatment. Both in terms of HIV-1 specific responses as well as general immune responses. No differences in CD4+ T-cell count or in activation of CD4+ or CD8+ T-cells was detected, as measured by expression of CD25, CD38 or HLA-Dr. Nor did we detect any effect on HIV-1 specific responses against Gag and Nef peptide pools. However, a consistent increase in CD25+CD127+ regulatory T-cells was found in all subjects after IVIg treatment
We and others [142, 143] have thus shown increased levels of regulatory T-cells after IVIg treatment. This implies that IVIg treatment could not only be considered as a possible candidate for reducing the cell pool, but also as a complement to treatment during chronic infection to decrease immune activation. This could have beneficial effects on the pathogenesis that is seen during late stage disease. However, due to the high costs and the need of parenteral administration, it is less likely that this approach will a major candidate for immunological intervention.

Several compounds have been examined in possible strategies trying to reduce the pool of latently infected resting T-cells, and most of these attempts have suffered form drawbacks (see table in the introduction part).

We realize that these data should be handled with care and needs further investigation. The optimal condition to study a potential decrease in the HIV-1 reservoir would be to retrieve resting T-cells through leukapheresis in order to have a large number of input in the cell cultures that would ensure a large number of replicates. In our study, we did not have access to that method. Instead we purified cells from 180 ml of whole blood and retrieved approximately 20 million cells to culture and subsequently had a limitation in replicates possible. Still, the present study indicates that IVIg may have the ability to decrease the pool of latently HIV-1 infected CD4+ T-cells and our conlusion is supported by the transient increase in plasma viremia in connection to IVIg treatment, and the decrease of residual viremia at follow up.

Paper III

Study background

Deliberate activation of the latent infection combined with effective antiretroviral treatment may be the best strategy to combat latent HIV-1. However, there are multiple factors that contribute to HIV latency in resting CD4+ T-cells, and a more detailed understanding of the underlying mechanisms behind HIV-1 latency and persistens in reservoirs is necessary in order to develop new eradication protocols. [142, 143].
Such factors inhibit virus gene expression after integration into cellular DNA and several studies have emphasized the importance of the chromatin structure at provirus integration sites for repressing the transcription of the provirus REF. The goal with the current study was to screen cDNA libraries for genes that are important for the regulation of latency and to identify molecules that can reactivate latent HIV-1.

New regulators of HIV-1 latency

There are several obstacles in performing large screening studiesusing latently infected T-cells, e.g. due to the low number present and to the fact that they are not phenotypically distinct from other resting cells. Therefore, a J-Lat cell line was constructed to harbor full length HIV-1 genome, similar to latently infected CD4+ T-cells. This latent provirus encodes the GFP gene, providing a fluorescent marker of HIV-1 transcriptional activity. After construction, a screen of cDNA in J-Lat cells was performed to search for genes that reactivate latent HIV-1.

The cDNA screen identified a portion of methyl-CpG binding domain protein 2 (MBD2), a transcriptional repressor that binds methylated DNA. The HIV-1 promotor was found to be hypermethylated in J-Lat cell lines and in primary CD4+ T-cells at two CpG island surrounding the HIV-1 transcriptional start site. Furthermore, inhibition of the provirus methylation lead to reactivation of the latent HIV-1 in the L-Lat cell line when combined with the activators of NF-kB.

Aza-CdcR and NF-kB activators synergistically induce HIV-1 replication in J-lat cells and primary CD4+ T-cells

The most important finding was that a small molecule inhibitor of DNA methylation, 5áza-2´deoxycytidine (aza-CdR) synergizes with NF-kB activators to promote an almost complete reactivation of latent HIV-1 in the J-Lat cells. These data are in line with the model that sequence specific transcription factors, including NF-kB, and cytosine methylation cooperates to maintain HIV-1 latency. NF-kB signaling can be adequate to reactivate latent HIV, but not to a full extent, indeed other factors are needed for complete activation [144-150]. Such as DNA methylation inhibitors.

The association between HIV-1 latency and cytosine methylation we found in the J-Lat cell line was confirmed in experiments using a primary model of HIV-1 latency. Here we infected PBMCs from healthy blood donors, and the results from the J-Lat experiments were confirmed.

We also initiated attempts to reactivate latent virus from the resting memory CD4+ T-cell pool from aviremic HIV-1 infected patients (data not shown). We detected reactivation of HIV-1 when adding methyl inhibitor Aza dC in combination with the transcription activatror Prostratin. However, we experienced problems with reproducibility in these directly *ex vivo* experiments as compared to the controlled J-Lat cell line model of HIV-1 latency. This was likely due to technical issues since the input of replication competent HIV-1 was very small, only about one per million resting T-cells, and the cell input to the cultures were two millions. . Furthermore, Aza dC interacted with other compounds in a stimulatory way, while being toxic effect on the primary T-cells when added alone. Therefore we did not pursue with the experiments and the results were not included in the manuscript..

However, Aza-CdR is approved for use in humans to treat myelodysplastic syndrome why it should be possible to investigate the effect also in HIV-1 infected patients. The addition of cytosine methylation inhibitor and NF-kB activators, such as Prostratin, to enhance the effect antiretroviral therapy could therefore be considered as a possible strategy towards eradication of latently infected cells in HIV-1 infected patients.

Paper IV

Study background

HMGB1 has been a major interest in our group for several years and we have earlier shown that this molecule may activate latent HIV-1 replication in vivo [151]. However, it seems that HMGB1 does not have high proinflammatory action on its own [152] , instead it may form complexes with other molecules, such as Toll like receptor ligands, and thereby become a more potent mediator of inflammation or cell activation [153, 154].
Immune activation and microbial translocation have been proposed to play a central role in HIV-1 infection [56-58, 155]. The preference for HIV-1 to infect activated CD4+ T-cells, as well as structural defects to the gut caused by the virus, may lead to translocation of microbial products such as LPS, flagellin and CpG DNA. These products are ligands for Toll like receptors and have the ability to activate the immune system [156].

The aim of this study was to explore if, and to what extent, complexes of HMGB1 and the TLR ligands LPS, CpG DNA and flagellin could induce HIV-1 replication in chronically infected cells.

HIV-1 replication is induced by complexes of HMGB1 and Toll like receptor ligands in U1 cells

We exposed U1 cells, a persistently infected promonocytic cell line, to necrotic extract, HMGB1 depleted extract and PMA, respectively. We found a two fold increase of viral replication in the supernatants with necrotic extract compared to the depleted extract and mock cells, as measured by p24 antigen concentration.
After we had seen the effect of necrotic extract on our cell cultures we wanted to test the effect of HMGB1-TLR ligand complex constructs. Therefore LPS, flagellin, CpG ODN and IL-1 b, respectively, were incubated with HMGB1 to enable complex formation and added to the cell cultures. We also tested each of the compounds separately. Stimulation with LPS, CpG, and IL-1 b in complex with HMGB1 resulted in a 1.5 – 2 fold increase of viral replication compared to the compounds alone, and for the HMGB-flagellin complex the increase was 7-fold.

To ensure that the stimulating effect could be linked to HMGB1, we repeated the experimental set up with recombinant HMGB1 in complex with the TLR ligands. The viral replicaton was increased in this set up as well, although not to the same extent as for the complexes with necrotic extract.

We also incubated U1 cells with TLR5 antibodies to block the effect induced by flagellin and detected a dose dependent inhibition of HIV-1 replication.

Our data show that the necrotic extract in complex with TLR ligands were more potent triggers of HIV-1 replication than the constructs with recombinant HMGB1. This is not a surprise since other stimulatory signals is likely to be derived from a necrotic cell extract. It could be argued that there is an uncertainty to what extent HMGB1 from necrotic extract in complex with TLR-ligand contributes to increased replication. However, the reduction of the stimulatory effect by depletion of HMGB1 strengthens our hypothesis that the molecule has a central part in this process.

Elevated levels of flagellin-specific antibodies in HIV-1 infected patients

We were intrigued by the *in vitro* flagellin data and wanted to explore the significance of this bacterial product further. Flagellin specific antibodies were measured from serum drawn from six HIV-1 infected patients and from three controls. In all of the HIV-1 infected patients, increased levels of anti-flagellin antibodies were detected compared to the controls.

Our *in vitro* and *in vivo* findings are clearly in line with the hypothesis of an important role of flagellin in HIV-1 pathogenesis. Here we have shown that flagellin in complex with HMGB1 are able to stimulate HIV-1 replication in persistently infected cells. We also demonstrate presence of anti-flagellin antibodies in serum from HIV-1 infected patients which indicate *in vivo* implications. The elevated adaptive immune response to flagellin has been observed previously in conditions associated with gut barrier dysfunction such as Crohn disease and short bowel syndrome [157, 158] and related to severity of Crohn disease [159, 160].
Our results deserve future studies of adaptive flagellin immune response in a larger cohort of HIV-1 infected individuals.

In conclusion, HMGB1 and the TLR ligands LPS, flagellin and CpG DNA forme active complexes that may induce viral replication in infected cells. We also report, that flagellin should be considered as a microbial product that can contribute to the immune activation during the HIV-1 infection. The formation of HMGB1/TLR ligand complexes may have direct implications on immune activation, particularly in late stage of disease, where cell destruction and necrosis are dominant phenomena due to CD4+ T-cell loss, opportunistic infections, and other pathological conditions.

Concluding remarks

- At initiation of our study resting memory CD4+ T-cells were recently reported to be important cellular reservoirs for HIV-1 in peripheral blood in patients with undetectable viremia during ART. In contrast, our data suggest that there is a turn-over of this cell-pool in untreated and sub-optimally treated patients and therefore archived founder or earlier resistant viral variants are not always found in these cells. We are however aware of the difficulties in trying to analyze the nature and dynamics of HIV-1 latency in the setting of low viremia due to the excess of unintegrated viral DNA. The kinetics of viral decay in resting memory CD4+ T-cells in viremic patients is therefore still a matter of discussion.

- It seems possible to reduce the pool of latently infected resting memory CD4+ T-cells in vivo, as suggested by our treatment of HIV-1 infected patients with intravenous immunoglobulin (IVIg) in addition to antiretroviral treatment. The IVIg treatment also resulted in an expansion of regulatory T-cells, which might have important implications for tampering immune activation in HIV-1 infected patients.

- Cytosine methylation may be an important component that controls HIV-1 latency. The combination of the compounds aza-CdR and prostratin can reactivate latent HIV-1 *in vitro* and minimize additional HIV-1 infection and general T-cell activation.

- Recombinant HMGB1, or HMGB1 as part of necrotic extract , in complex with TLR stimulating ligands LPS, CpG ODN and flagellin, increased HIV-1 replication *in vitro,* as compared to HMGB1 or ligands alone, in a model of latent HIV-1 infection.

- Our *in vitro* experiments have identified potential approaches for activation of latent HIV-1 *in vivo*, and further exploration of the mechanisms should be considered.

Populärvetenskaplig sammanfattning

Humant immunbrist virus, HIV, är ett omfattande globalt problem och är ett virus som allvarligt skadar kroppens immunförsvar. Uppskattningsvis lever ungefär 33 miljoner människor med HIV idag och 2,5 miljoner dör till följd av infektionen varje år. Värst är situationen i Afrika, men antalet HIV infekterade ökar kraftigt i Ryssland, Östeuropa och Centralasien.

HIV infektionen läker inte ut spontant och är dödlig om den infekterade inte får behandling. Nedbrytningen av immunförsvaret är en långsam process och utan behandling tar det i genomsnitt 10 år från den primära infektionen tills den infekterade utvecklar AIDS, vilket är slutstadiet av en HIV infektion. AIDS kännetecknas av att immunförsvaret inte längre kan skydda kroppen mot olika bakterier, virus, svampar och parasiter som normalt inte skulle utgöra något större hot för en frisk person.

De mediciner som finns till hands är så kallade bromsmediciner och de sätts in redan när immunförsvaret är lite försvagat. Medicinerna kan inte bota infektionen, men de kan kontrollera den virusmängd som finns i kroppen och därmed också bromsa nedbrytningen av immunförsvaret.

Trots att HIV kan behandlas med gott resultat kvarstår problemet att viruset faktiskt finns kvar i kroppen. HIV kan lagras i kroppens arvsmassa och därmed etablera en ”latent”(sovande) infektion. Det beror på att HIV föredrar att infektera en typ av celler som kallas vilande minnesceller. De är mycket långlivade och har som sin viktigaste uppgift att dirigera andra komponenter i vårt försvar mot främmande ämnen. Utan dem brakar immunförvaret samman.

I den här avhandlingen ingår fyra delarbeten där jag har varit fokuserad på att studera olika aspekter av den latenta HIV infektionen.

I det första delarbetet undersökte vi om ursprungliga och resistenta virusstammar kunde återfinnas i vilande minnesceller hos obehandlade patienter, samt hos patienter som fått en behandling som inte varit optimal, dvs där patienten utvecklat resistens mot en eller flera bromsmediciner. Vi jämförde ”fritt” virus ifrån blodplasma med virus som integrerats i vilande minnesceller för att se hur lika/olika virusstammarna var.
Det är känt att olika virusstammar från olika tidpunkter kan arkiveras i vilande minnesceller, men de stammar vi hittade var mycket homogena vilket antyder att det sker en omsättning av viruset i de här två patientkategorierna.

I det andra delarbetet aktiverade vi vilande minnesceller hos välbehandlade HIV infekterade patienter med intravenöst immunoglobulin, IVIg, för att se om det kan vara en metod för att minska andelen HIV infekterade vilande minnesceller. Vi arbetade med en metod som gör det möjligt att mäta hur många av dessa celler som är infekterade med HIV. Mängden HIV i minnesceller mättes före och efter IVIg behandling och vi fann en minskning av HIV infekterade minnesceller hos en majoritet av patienterna.

I det tredje arbetet undersökte vi en möjlig mekanism som bidrar till kontroll av den latenta HIV infektionen i minnesceller. Många försök har gjorts där man velat aktivera latent virus i kroppen med förhoppning om att få ut arkiverat virus, men ännu har inget lyckats helt. Det krävs därför ytterligare kunskap om vilka cellulära och virala faktorer som håller cellen, och därmed viruset, vilande. Vi fann att man med hjälp av vissa ämnen kan påverka cellulära faktorer som direkt interagerar med arvsmassan i cellerna. Genom att tillföra dessa ämnen kan cellens maskineri komma igång och viruset aktiveras.

I det fjärde arbetet ville vi undersöka några faktorer som kan bidra till den ”överaktivering” av immunförsvaret man kan se hos HIV infekterade. En kandidat till detta tror vi är HMGB1, ett protein som finns i cellkärnan i alla celler i kroppen. Proteinet har en viktig roll vid inflammatoriska processer i kroppen, och vår forskargrupp har tidigare visat att HMGB1 är en viktig faktor i sjukdomförloppet vid HIV-infektion. Vi gjorde mixar av HMGB1 och bakteriella ämnen som vi vet påverkar aktivering av immunförsvaret. Försöken gjordes på celler som är en modell för latent HIV-infektion. Vi fann att HMGB1 tillsammans med bakteriella komponenter ökade immunaktiveringen i betydligt högre utsträckning än vad de olika ämnena gjorde var för sig.

Den latenta HIV infektionen i vilande minnesceller utgör det största hindret för att kunna bota HIV infekterade och det behövs fortfarande mer kunskap om vilka mekanismer som kontrollerar detta. Jag hoppas att de arbeten som presenterats i den här avhandlingen kan vara ett litet bidrag och ett steg i rätt riktning .

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to the people that directly or indirectly contributed to this work, especially to:

My supervisor: Anders Sönnerborg for the opportunity to be a part of "Team Sönnerborg" for all these years. For your friendship, guidance, encouragement and generosity.
I´m truly grateful for the opportunities to attend international conferences , it´s been a great opportunity to see the world.
And last but not least, for your patience…
.
My co-supervisors for all your help and support:
Annika Karlsson for your guidance and especially for the initial introduction to the lab and labwork at F68. And for becoming a good friend through the process . =)
Piotr Nowak for introducing me to, and helping me through, a part of your HMGB1 project. Even though it (I) seemed liked "mission impossible" sometimes…

Professor Anders Vahlne for giving me the opportunity to work at Clinical Virology, now Clinical Micobiology.

All the patients at Karolinska University Hospital and Sahlgrenska University Hospital who participated in the projects and made the work possible.

Alenka, for your friendship and for all the nice lunches. "We will eventually make this a better world to live in." ;) And also a special thanks for your support during these last weeks… Don´t now what I would have done without you…

Eva, for being a great friend and for all the nice chats during the years

Forskarskolan i Vård och Omsorg

Övriga co- authors (also to the ones from the papers not included in the thesis)

Andrej for patience with my seminars…

Kerstin and Gudrun for all your (ovärderlig!) help with paperwork etc, and for your nice encouraging words

And Kerstin, thanks for being a good friend and for all the Theater tickets you´ve supplied during the years (often including your company =))

The present and former nurses at Infektionsmottagning 2, especially Marja, Sofia and Margit.

All the students and staff at ”Labbet”

Former and present members of team Sönnerborg (Eva, Jessica, Irene, Samir, Amanda, Babbi, Piotr, Marius, Tobias, Halime, Markus, Soo mfl)

F68 Klinisk Virologi, with special thanks to Charles, Anne Q, Eileen, Lisa , Hamzah and the staff at HIV/Hep. And a thanks to Ulla (who is no longer with us) for all your help and nice chats in P3.

“Frukostklubben”

Lillian for help with Luminex

Hernan for helping out with FACS work

A great big thanks to the computer guys, Fredrik and Tore, for all the assistance with my computer. Two days before I was going to hand in my thesis, my computer(s) was infected …

To Robert and Sune at reproprint for giving “flexibility” a new dimension…

My dear, dear friends Christel, Mia, Joanna and Malin. Thank´s for being around all these years! (“Omständigheter” kommer och går, men kärnan består…) Can´t think of a “Midsommar” or “Nyår” without you!!!

My Family, in particular mormor who is the strongest women I´ve ever met…

Jan for your help and support. Especially for your outstanding performance as a lab technician when our incubator was contaminated… I was really impressed… ;)

Harry, for your soothing effect…

And finally, the two main reasons for making life worth living, my beautiful sons Christoffer and Jonathan. You are my sunshine and I love you with all my heart!

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