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Aspects of immune activation in HIV-1 infection

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Stockholm 2007

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Sundbyberg, Stockholm, Sweden
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ISBN 978-91-7357-190-6

To my family

Abstract

Abnormal immune system activation is central in HIV-1 pathogenesis leading to CD4+ T-cell depletion and inappropriate immune responses. Disease stage and HIV load strongly correlate with the immune activation level which decreases under antiretroviral therapy (ART).

The general aim was to investigate the role exerted by immune activation in HIV-1 infection and to characterise the contribution of high mobility group box protein 1 (HMGB1). This was fulfilled by: (a) studying two unique cohorts consisting of four orthotopic liver transplant (OLT) patients receiving ART and immunosuppressive therapy, and 22 patients with early primary HIV infection (PHI) who underwent both ART and analytical treatment interruption (ATI); (b) characterising the interaction between HMGB1 and HIV-1 *in vitro* and *in vivo*.

HIV-1 was controlled by ART, without any major contribution of the adaptive immunity, as shown by low or undetectable viral load (VL) and restricted viral diversity-divergence, in the four OLT patients. Also, the kinectics of VL during PHI in an OLT subject suggested that the initial rapid viral decline is caused by the innate immunity or reduced immune activation. Furthermore, the lower degree of immune activation in 22 PHI-patients was associated with lower viral replication and improved long-term virological outcome, as implied by the kinetics of 13 cytokines and chemokines during PHI and post ATI. We showed that PHI is indeed associated with generalized immune activation not restricted to a specific cytokine/chemokine pattern.

HMGB1 is an abundant protein with intracellular localisation and function exerting DNA flexibilisation and regulation of transcription. Additionally, this protein can be released actively after activation of macrophages, and dendritic cells, and passively by necrotic cells. Exogenous HMGB1 is a proinflammatory mediator that has been attributed a plethora of functions. Our data indicate that HMGB1 has an important role during HIV-1 pathogenesis. Thus, intracellular HMGB1 could inhibit the LTR mediated HIV-1 transcription in epithelial and monocytic cells. The physiological overexpression of HMGB1 in myeloid cells might be responsible for the restricted HIV-1 replication in these cells. Moreover, extracellular HMGB1 had a regulatory function on HIV-1 replication, tested in two models, latent and acute. We found upregulation of viral replication after HMGB1 stimulation of U1 cells and downregulation in acutely infected primary macrophages. The latter effect could be explained by β-chemokines (MIP-1α, MIP-1β, RANTES) release from these cells after HMGB1 induction. ELISA analysis revealed that the plasma levels of HMGB1 are significantly elevated in HIV-1 infected individuals (n=43) as compared to HIV-1 negative individuals (n=14). Additionally, the HIV-1 infected patients with clinical complications (n=16) had higher levels of HMGB1 than asymptomatic patients with preserved (n=14) or deteriorated immune system (n=13).

In conclusion, the data included in this thesis stress the essential role of immune activation in HIV-1 pathogenesis and place HMGB1 as an important factor that can modify the HIV-1 replication and contribute to the dysregulation of the immune system.

Keywords: immune activation, HMGB1, PHI, HIV-1 LTR-transcription, cytokines, chemokines

List of Publications

I. **Nowak P**, Schvarcz R, Ericzon BG, Flamholc L, Sönnerborg A.
Follow-up of antiretroviral treatment in liver transplant recipients with primary and chronic HIV type 1 infection.
AIDS Res Hum Retroviruses. 2003 Jan 1;19(1):13-9.

II. Naghavi M. H, **Nowak P**, Andersson J, Sönnerborg A, Yang H, Tracey K J. and Vahlne A.
Intracellular high mobility group B1 protein (HMGB1) represses HIV-1 LTR-directed transcription in a promoter – and cell –specific manner.
Virology 2003, Sep 15; 314 (1): 179-189

III. **Nowak P**, Barqasho B, Treutiger CJ, Harris HE, Tracey KJ, Andersson J, Sonnerborg A.
HMGB1 activates replication of latent HIV-1 in a monocytic cell-line, but inhibits HIV-1 replication in primary macrophages.
Cytokine 2006 Apr;34(1-2):17-23.

IV. Barqasho B, **Nowak P**, Tjernlund A, Kinloch S, Goh L-E, Lampe F, Fisher M, Andersson J, Sönnerborg A.
Kinetics of plasma cytokines and chemokines during primary HIV-1 infection and after analytical treatment interruption. Submitted 2007.

V. **Nowak P**, Barqasho B, Sönnerborg A.
Elevated plasma levels of high mobility group box protein 1 in pateints with HIV-1 infection. *AIDS* 2007, Apr 23, 21(7): 869-71

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List of abbreviations

AIDS	Acquired Immune Deficiency Syndrome
APOBEC3G	Apolipoprotein B mRNA–Editing Enzymes
ART	Anti Retroviral Therapy
CCR	CC chemokine receptor
CMV	Cytomegalovirus
CTL	Cytotoxic T Lymphocyte
CXCR	CXC chemokine receptor
EBV	Epstein Barr Virus
HAART	Highly Active Anti-Retroviral Therapy
HIV-1	Human Immunodeficiency Virus 1
HIV-2	Human Immunodeficiency Virus 2
HMGB1	High Mobility Group Box protein 1
IL	Interleukin
LTNP	Long Term Non Progressor
LTR	Long Terminal Repeat
MCP	Monocyte Chemotactic Protein
MDM	Monocytes Derived Macrophages
MIP	Macrophage Inflammatory Protein
mRNA	Messenger RNA
NFAT	Nuclear Factor of Activated T cells
NF-κβ	Nuclear Factor Kappa B
NK	Natural Killer Cell
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PHI	Primary HIV-1 Infection
RAGE	Receptor for Advanced Glycation Endproducts
RANTES	Regulated on T cell Activation, Normal T cell Expressed and Secreted
RT	Reverse Transcriptase
SIV	Simian Immunodeficiency Virus
TAR	Tat-Responsive Element
TLR	Toll-like Receptor
TNF	Tumor Necrosis Factor
UNAIDS	Joint United Nations Program on HIV/AIDS
WHO	World Health Organization

Introduction

Despite the increased knowledge and available chemotherapy HIV remains a global health treath. The epidemic is still successfully spreading with 15 000 people being infected every day, particularly in low to middle income countries. In the end of 2006 40 millions people have been infected with HIV [1]. The virus can not be eradicated even with successful treatment and the preventive vaccine is far from the pipe-lines of pharmaceutical companies. Even if previous sentences are not encouraging, substantial progress has been made both in the field virology and immunology since the discovery of HIV. This advancement in the understanding of the viral pathogenesis and the interactions with the immune system gives us the hope for the future combat against the HIV.

The work included in this thesis has focused on the role of immune system activation in HIV-1 infection. Particular emphasis has been placed on the characteristics of the high mobility group box protein 1 (HMGB1) that we believe is important for this process.

The epidemic

In 1981 a new clinical syndrome, the acquired immunodeficiency syndrome (AIDS) was first described, after the identification of an increased incidence of opportunistic diseases in previously healthy young homosexual men [2-4]. The first indication that AIDS could be caused by a retrovirus came in 1983, when the agent was isolated in France from a patient with a pre-AIDS syndrome [5]. The virus was given the name lymphadenopathy virus (LAV). Researchers in USA isolated an identical virus the year after and named it AIDS associated virus [6] or human T-cell lymphotropic virus (HTLV III) [7].

The virus was later given the name human immunodeficiency virus (HIV). In 1986 a second immunodeficiency virus was discovered [8], named HIV-2, and the former strains of HIV were referred to as HIV-1. HIV-1 and HIV-2 are lentiviruses belonging to the *Retroviridae* family [9]. These viruses were found to be spread by sexual contacts, parenteral contact including blood and by vertical transmission from mother to child. HIV-2 also causes AIDS but appears to be less infectious and pathogenic than HIV-1 [10].

HIV infection has spread to all continents and has been responsible for the death of 25 million people. Approximately 4.3 million people became infected and 2.9 million people died due to HIV/AIDS only during year 2006 (www.unaids.org).

Virological aspects of HIV-1 infection

Virion structure

The HIV-1 virion is about 110 nm in diameter and has a spherical shape [9]. The envelope is a host derived lipid bi-layer which contains anchored viral trimeric structures consisting of surface (gp120) and transmembrane (gp41) subunits bound non-covalently [11]. The glycoprotein gp41 is attached covalently to the matrix (MA) p17 protein that lines the inner surface of the virus envelope, surrounding the nucleocapsid complex [12]. As an immature virus, the HIV-1 core has a doughnut-like structure which is then transformed into cone-shaped structure upon maturation as a result of Gag processing [13]. The capsid core contains the viral genome which are two copies of single stranded RNAs tightly bound to the nucleocapsid (NC; p7) protein. Other viral proteins contained here are the reverse transcriptase (RT; p66/p55), the protease (PR; p15) and the integrase (IN; p32). A schematic picture of HIV-1 can be seen in figure 1.

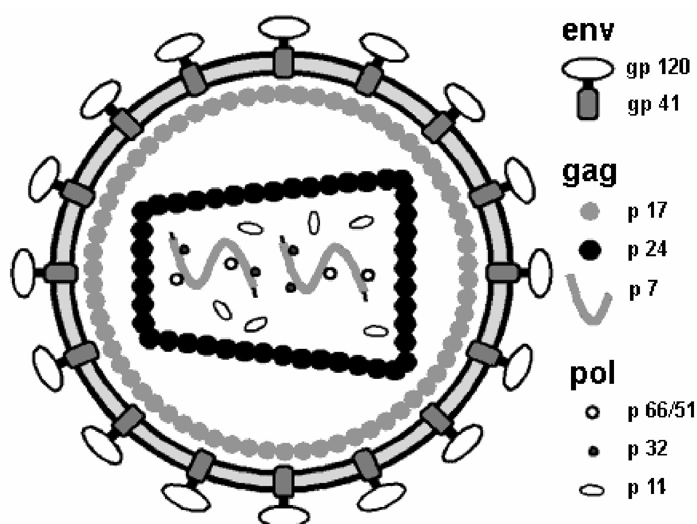

Figure1. Schematic overview of HIV-1 structure

Genomic structure and replication

The HIV-1 genome is approximately 9200 bases in length [9]. In common with all replication competent retroviruses, the HIV-1 genome has three major structural genes (*gag, pol, env*), which are flanked on both sides by repetitive sequences called long-terminal repeats (LTR) [14]. The *gag* gene encodes the structural proteins: capsid (p24), matrix (p17) and nucleocapsid (p9/p7). The *pol* gene encodes reverse transcriptase, integrase and protease. These proteins are essential enzymes for transcription of viral RNA into DNA, integration of viral DNA into the cell genome and cleavage of HIV-1 proteins, respectively. The *env* gene codes for the envelope

glycoproteins (gp120 and gp41) that are critical for viral attachment and subsequent infection of the target cell [15]. Beside the three structural genes, the HIV-1 provirus contains six additional open reading frames, coding for the regulatory proteins (Tat, Rev) and the accessory proteins (Vif, Vpr, Vpu and Nef) which functions are of importance for viral replication and infection (Table 1) [16, 17].

Table 1. Additional proteins of HIV-1 and their functions.

Gene	Product	Function
vif	p23	enhances infectivity of virions/ counter-acts APOBEC3G
vpr	p15	involved in targeting the nuclear localization of preintegration complexes; arrest cell cycle at G2 phase
vpu	p16	degradates CD4 in the endoplasmic reticulum; assists budding
tat	p14	potent transactivator
rev	p19	RNA transport and stability; regulation of viral gene expression
nef	p27	interferes with endosome trafficking and downregulates expression of CD4 and MHC-1; effect on cell activation / apoptosis induction in bystander cells

The HIV-1 infection of a cell is initiated by interactions between the viral envelope glycoprotein (gp120) and the cellular receptor, the CD4 molecule (Figure 2) [15]. Thus, the major targets for HIV-1 are cells expressing the CD4 antigen on their surface. These cells include T-lymphocytes, monocytes, macrophages, and dendritic cells [18-20]. After binding to the CD4 molecule, gp120 subsequently interacts with one of the coreceptors of the chemokine receptor family (CCR5 and/or CXCR4) [21]. Also other chemokine receptors have been shown to be functional as coreceptors in the viral entry, although their relative importance seems to be limited [22]. This interaction results in a conformational change of gp41, which in turn leads to a pH-independent fusion between the virus envelope and the cell membrane [23]. After that the fusion is accomplished the virus is unpackaged and the nucleocapsid is dismantled. The reverse transcriptase then transcribes viral RNA into DNA, which subsequently enters the cell nucleus in the form of a preintegration complex [24]. The proviral DNA is then incorporated into the host cell DNA by the viral integrase [25]. Frequently transcription of the integrated proviral DNA is initiated immediately, but in some cells the proviral DNA remains latent for a variable length of time until host cell activation [26]. The cellular activation initiates transcription of the structural genes into mRNA, which are then transported out of the nucleus into the cytoplasm [27]. Following translation, the viral proteins begin to assemble at the host cell surface which is modified by insertion of gp41 and associated gp120 [28]. The viral RNA and the core proteins assemble beneath the modified membrane, acquiring the modified host plasma membrane as its envelope during the budding process [29]. The viral Gag and Gag-Pol polyproteins are cleaved by the viral protease during or shortly after budding, generating mature infectious virons [30].

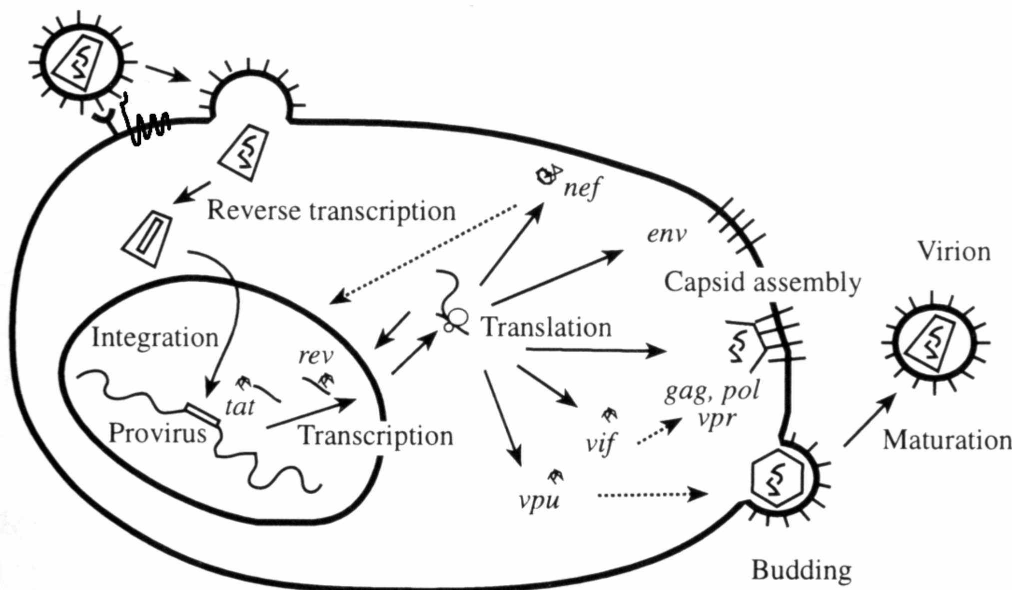

Figure 2. The HIV-1 replication cycle.

LTRs and HIV-1 transcription

The long terminal repeats (LTRs) sequences contain promoters, enhancers and other sequences that interact with host-cell transcription factors as well as virus encoded proteins and modulate expression of viral RNA and proteins [31]. LTR function has a direct impact on the productive infection of the host cell, viral expression and assembly, production of cytotoxic viral proteins and the transition from latency level of viral expression to highly productive infection [32]. Although, both the 5' and 3' LTRs facilitate integration of the viral genome into the host chromosome these two LTRs are distinct in function. The 5' LTR functions as a promoter/enhancer and therefore directs HIV-1 transcription, whereas the 3' LTR is required for cleavage and polyadenylation of the viral mRNAs [33].
The LTR extends from -454 to +184 (related to the site of transcription) and is structurally divided into three regions: the 454 bp U3 region, the 97 bp R region and the 85 bp U5 region [31]. Two nucleosomes have been positioned in the 5'LTR regions: the first one located in the 5' of the U3 region (nuc-0) and the second one immediately after the transcription initiation site in the R region (nuc-1) [34]. These sites define two large nucleosome-free regions encompassing upstream and downstream of the site of transcription initiation. After initiation of viral transcription, nuc-1 is disrupted and an additional hypersensitive site appears in the R region of the 5' LTR. Thus the U3-R part of the HIV-1 LTR is associated with an open chromatin configuration which point to the regulatory role of this region in the viral gene expression. The U3-R part contains cis-acting sequences and may be subdivided into several elements due to function during the transcription: the modulatory (-454 to -104), the core enhancer (-105 to -79), the core promoter (-78 to -1) and the Tat Responsive Element termed TAR (+1 to +60) (Figure 3). U5 region

also contains several constitutive and inducible regulatory elements that are important for efficient HIV-1 transcription and replication [32, 35].

HIV-1 LTR-directed transcription is RNA polymerase II (Pol II) regulated by the complex interactions of host transcription factors and viral proteins, with specific DNA as well as RNA sequences on the HIV-1 5'-LTR [31]. The initiation of viral transcription occurs in the core promoter which contains the key regulatory elements required for transcription. These sites include: three SP-1 binding sites, a conserved TATA box and an initiator element (Inr) [36-38]. The presence of these three elements is crucial for minimal promoter activity *in vitro* and *in vivo* [39, 40]. The LTR contains two initiator elements. Several different cellular factors have been identified to interact with initiator elements and positively or negatively regulate HIV-1 basal transcription [35]. The enhancer region consists of two tandem binding sites for the transcription factor NF- κβ. Several proteins members of the Rel family can bind to these sites, associating as homo or hetero-dimers [41]. This binding to NF- κβ motifs in HIV-1 enhancer increase expression of HIV-1 proteins in T-cells and macrophages [42]. However the NF- κβ sites are not absolutely required for viral replication and appear to serve primarily to enhance viral replication in response to cellular activation and differentiation [43, 44]. It has been shown that NF- κβ can functionally compensate for Sp1 in activating HIV replication [45]. Several cellular, constitutively expressed or inducible host-cell transcription factors, which bind to the modulatory element within the LTR have been identified [32]. This element contains a negative regulatory region (NRE) of about 260 bp sequences. NRE despite its name confers also positive regulation and contains binding sites for many cellular transcription factors including: USF, hLEF and NFAT [46, 47].

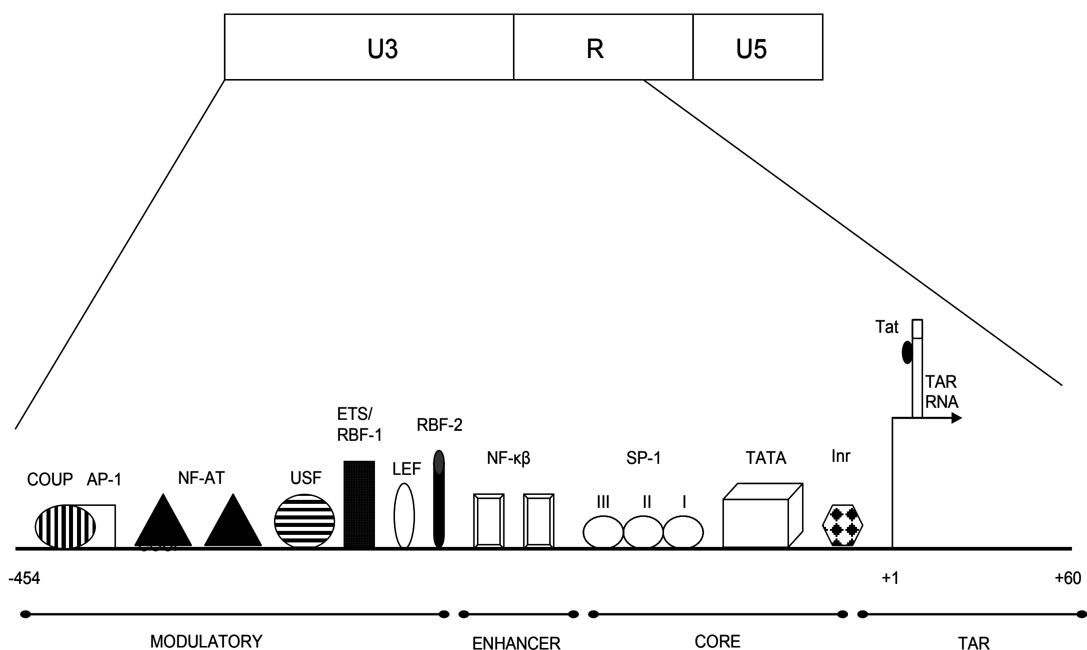

Figure 3. Schematic diagram of the HIV-1 5'-LTR. The U3 and R regions are divided into the TAR, core promoter,enhancer and modulatory elements. Binding sites for the identified cellular transcription factors are indicated. Modified from [31].

The early phase of HIV-1 transcription is regulated by cellular transcription factors and results in the production of early viral products (Tat, Rev and Nef) [48, 49]. The late phase of transcription is under control of Tat which potently enhances gene expression by a direct binding to TAR-RNA and association with cyclin T1 (Cyc T1), which recruits the cyclin dependent kinase 9 [50]. This leads to phosphorylation of the C-terminal domain of RNA polymerase II and efficient elongation. Tat can also stimulate transcriptional initiation and it has been shown that Tat can synergize NF-kβ activity [51, 52]. These two steps of provirial transcription occur in various cell types, which indicate that distinct combination of factors mediate transcription in a cell type-specific manner [31, 49].

Variability and heterogeneity

HIV-1 is characterized by a remarkable genomic heterogeneity [53]. HIV-1 genetic variants appear readily in individuals, which result in a swarm of closely related genomes termed 'quasiespecies' [54]. The rapid evolution of HIV-1 and the high genetic diversity are results of the low fidelity of the reverse transcriptase (RT), recombination events, selective host immune responses and the high turnover rate of virus production. The low fidelity of the RT is caused by the lack of the 3'exonuclease proof-reading activity, which makes it very error prone, and thus allows for nucleotide substitution, deletions, insertions, duplications and recombinations [55, 56]. The rate of nucleotide substitution caused by the RT is about one substitution per virus genome per cycle of replication [53, 57]. Also, host RNA-polymerase II, responsible for viral genomic RNA synthesis, is lacking 3'exonuclease proof-reading activity. This high error rate in combination with the fact that in HIV-1 infected individuals approximately 10^{10} new virus particles are produced and cleared per day with a life span in cells about two days results in the high viral diversity [58]. The sequence variation is due to nucleotide substitutions that are either synonymous (d_s), which do not change the amino acid, or nonsynonymous (d_n) that cause changes. The d_s/d_n ratio varies between different genomic regions and is in part influenced by the selective pressure of the immune system [59, 60]. Products of the *pol* gene are essential for viral replication, which is the reason why this region shows limited variability. The largest variation occurs in the *env* gene. This is illustrated by the fact that the gp120 part of envelope polyprotein has evolved to contain hypervariable domains that can accept extensive variation, thereby escaping from the host immune system. This region can thus be used as an indicator for viral evolution [61, 62]. The gp120 is divided into five variable (V1-V5) and five constant (C1-C5) regions [63]. The V3 region of *env* has attracted most attention. It is known that this region is a major target for humoral and cellular immune responses [64-66]. Changes of certain aminoacids within the V3 loop result in changes of the phenotype of the virus [67, 68], e.g in the use of co-receptors [21]. The variability in the p17 region of the *gag* is higher than in *pol,* but lower than in *env*. Because of these properties and the higher rate of d_s the p17 region has been proposed as being a useful marker for epidemiological studies [69]. The phylogenetical studies have revealed the dynamics of the viral population in the infected host. Thus, early after infection, the virus population is usually relatively homogenous [70-72]. During the disease progression, in the asymptomatic period the population of viral variants becomes more

heterogeneous probably as a result of continuous immunological selection [73-75]. A comprehensive study with frequent sampling from PHI up to twelve years defined three distinct phases within this interval: an early phase of variable duration with a linear increase of both viral divergence and diversity (~ 1% per year); an intermediate phase of mean 1,8 year with a continuous increase in divergence but stabilizing or declining diversity; and finally a slowdown or stabilization of the viral divergence and declining diversity [76]. In support of these data several reports show that, at the AIDS stage, the virus population returns to a relative homogeneity, due to lack of the pressure from damaged immune system [53, 77].

Natural course of HIV-1 infection

The acute infection with HIV-1 may be associated with clinical symptoms of variable severity and duration [78, 79]. Those symptoms usually occur within two weeks after viral entry [80]. The viral load (that can be detected around a week after infection) rapidly increases to reach the peak about one week after the onset of symptoms. The increase of viral load inversely correlates with CD4+ T-cell counts, depletion of which has been claimed to be caused mainly by direct virus effect [81]. The viremia peak is followed by a phase of rapid decay and subsequent phase of slow decay that starts after about 3 weeks after onset of symptoms [82]. The viral load reaches the steady-state 3 to 4 months after infection. It is believed that events occurring during the acute/primary phase of HIV-1 infection (PHI) determine the disease prognosis [83-85]. A correlation between the peak of viremia and viral set point as well as disease progression has been shown [86]. The chronic phase of infection, usually asymptomatic, can last for many years without a need for therapeutic intervention [87]. However, a continuous viral replication and gradual loss of CD4+ T cells lead to dysfunction of the immune system and the appearance of opportunistic conditions associated with AIDS, the final stage of HIV-1 infection [88, 89].

Opportunistic conditions include:

- Candidiasis of esophagus, bronchi, trachea, or lungs
- *Mycobacterium tuberculosis* (TB),
    - pulmonary
    - extrapulmonary
- *Mycobacterium*, other species, disseminated or extrapulmonary
- *Pneumocystis jirovecii* pneumonia (PCP)
- Pneumonia (other than PCP), recurrent
- Cryptosporidiosis, chronic intestinal
- Cytomegalovirus retinitis
- Toxoplasmosis of the brain
- Encephalopathy, HIV-related (AIDS dementia complex)
- Histoplasmosis, disseminated or extrapulmonary

- Kaposi's sarcoma (KS)
- Lymphoma: non-Hodgkin's (NHL), primary brain/CNS
- Progressive multifocal leukoencephalopathy (PML)
- Invasive cervical cancer

Treatment of HIV-1 infection

Drugs that are approved as antiretroviral agents target either the viral entry (introduced recently), reverse transcriptase, protease or integrase enzymes (currently introduced) [90-92]. A combination of these drugs is called highly active antiretroviral treatment (HAART) and is very efficient in reducing the HIV-1 viral load as well as immune activation [93, 94]. Morbidity and mortality have been dramatically decreased after HAART introduction [95]. However, HAART is unable to eradicate HIV-1 because replication competent viruses persist under therapy in an integrated latent form and/or in a slowly replicative manner in several cell types and anatomical sites [96, 97]. Moreover suboptimal ART, which allows the viral replication to continue, is known to select for virus variants with reduced sensitivity to the antiretroviral agents and drug-resistance is therefore a common problem [98]. From the patient perspective HAART although effective may have side effects such as neuropathy/myopathy (due to mitochondrial damage), gastrointestinal intolerance, a metabolic syndrome including lipid abnormalities and diabetes mellitus, a body change syndrome (lipodystrophy) and a slightly increased risk for myocardial infarctions [99, 100]. The appearance of long-term side effects is why alternative strategies such as structured treatment interruptions (STI) came into focus in the beginning of 2000s [101, 102]. These strategies have given the opportunity for studying the interactions of HIV-1 with immune system under monitoring conditions. The recently published results of SMART trial may change the approach to wider usage of intermittent therapies [103]. This open-label study assigned more than 5400 HIV-1 infected patients who were divided into the group receiving continuous or intermittent (CD4+ T-cell guided) ART. The episodic ART significantly increased the risk of opportunistic disease or death from any cause as compared with continuous ART. These findings have lead to early termination of SMART trial [104].

Immune activation

A complex combination of viral and host factors contribute to the pathogenesis of AIDS [15, 105]. The direct effects of the virus on T-cell death cannot explain the AIDS pathogenesis because of the low frequency of infected CD4+ T cells [106]. Additionally, virus preferentially infects activated CD4+ T cells, most of which are destined to die regardless of their infection, because of activation process itself [107]. An activation of the immune system is an important part of a proper immune response to pathogens. In general, after that the immune response adequately has dealt and cleared the foreign antigen, the system returns to the state of relative quiescence until the next stimulus is introduced. This does not happen in the case of

HIV-1 infection. The persistence of the virus leads to a constant exposure of the immune system to viral antigens and is likely to contribute to the chronic activation of the immune system observed in the HIV-1 infected patients [108]. The immune activation is the result of both the continuous attempt of the immune system to clear the infection and the HIV-1 associated dysregulation of its homeostasis. Thus, HIV-1 both directly (cell damage/induction) and indirectly (immune compensatory mechanisms) can contribute to immune activation [81]. This contributes the state of chronic inflammation, which may also be responsible for non-HIV-1 related complications, such as the increased rate of atherosclerosis in HIV-1 disease [109].

This process starts very early after PHI and persists throughout the whole disease course [110, 111]. The theory that HIV-1 causes T-cell dysfunction and T-cell loss by chronic immune activation could feasible explain AIDS pathogenesis [81, 112].

The study on chronic activation in a murine model [113] has elegantly showed that also in the absence of active infection the animals with hyperactive immune system could present signs of immunodeficiency syndrome with T-cell loss and opportunistic infections. Contrary, the lack of generalized immune activation in the natural hosts for SIV infection, sooty mangabeys, is associated with lack of pathogenicity despite high viral production and poor host immune response [114].

HIV-1 can infect both activated and quiescent cells, however the virus requires cell activation signals for genome integration as well initiation of its replication [27]. The *in vivo* connection between immune activation and HIV-1 replication is demonstrated by the increases in viremia in HIV-1 infected individuals transiently or persistently exposed to exogenous immune stimuli [115, 116]. Individuals living in Africa, chronically exposed to parasites and other pathogens, usually express higher levels of activation markers [117, 118] and harbour high viral loads [119] that are associated with faster disease progression [120]. In addition, co-infection with opportunistic pathogens results in significant increases of viral load, which decreases when the opportunistic infection is treated [121, 122]. Moreover, immunisation of HIV-1 infected patients with influenza or tetanus toxoid antigens, results in transient increase of plasma viremia [123]. In conclusion, immune activation is a requisite of HIV-1 infection, closely connected to the viral replication.

The immune activation can be characterized by many immunological signs including:

- increased T-cell turnover
- increased frequencies of T- and B-cells with activated phenotypes
- increased CD8+ T-cell counts
- increase in T-cell death rate apoptosis (AICD activation induced cell death)
- impairment of T-cell responses to antigens
- decrease of regulatory T-cells
- polyclonal B-cells activation-hypergammaglobulinemia, presence of autoantibodies
- activation of monocytes
- increased serum concentrations of proinflammatory cytokines and chemokines

Several studies have implicated that the levels of these markers represent independent predictors of disease outcome in antiretroviral-untreated and –treated individuals [124, 125].

Of these, the best characterized marker of immune activation is the CD38 expression on T-cells. CD38 is a multifunctional transmembrane glycoprotein that is up-regulated during the earliest stages of T-cell activation [126]. *Giorgi et al* [127] reported that CD38 expression on CD8+ T-cells had stronger prognostic value concerning HIV-1 progression than other commonly used markers of activation. However, even serial quantification of CD8+ T-cells gives significant predictive information of the natural progression of HIV-1 infection in patients with moderate to severe immunodeficiency [128]. An immune activation set point is established during PHI and predicts subsequent CD4+ T-cell loss, independent of VL [110]. Furthermore, chronic HIV-1 infection is associated with increased T-cells turnover [129]. Elevated markers of CD4+ T-cell apoptosis correlate with CD4+ cell depletion and virus production [130, 131]. Activation induced cell death (AICD) appears to be the major mechanism of uninfected ‘’bystander’’ T-cell loss due to direct action of viral proteins and inflammatory milieu [132]. Dysregulation of the cytokine network is characteristic of all phases of HIV-1 infection [133-135]. A number of cytokines have been shown to direct modulate HIV-1 replication in T-cells and macrophages *in vitro* and influence disease progression [134, 136]. Proinflammatory cytokines like TNF-α, IL-1β, and IL-6 are reported to be elevated during the primary and late phases of HIV-1 infection, contributing to immune activation and inducing apoptosis signals [136, 137]. β-chemokines, although exerting antiviral effects, can be also responsible for attraction of cells and contribute to viral spreading [138, 139]. Thus, diverging results about the levels of β-chemokines in relation to viral replication and disease progression are found in the literature [94, 140, 141]. Additionally, *Brenchly et al* have recently suggested [142] that microbial products originating from intestinal flora could contribute to immune activation. The authors reported that bioactive LPS is found in plasma of HIV-1 infected individuals with levels correlating to immune activation markers. Interestingly, the levels of LPS decrease with effective HAART and long-term non-progressors (LTNP) contain lower levels of this potent immune activator in plasma. The hypothesis that pathogenic events during PHI that lead to breach in the integrity of gastrointestinal mucosal barrier and cause microbial translocation is an attractive explanation of HIV-1 systemic immune dysregulation [81, 143].

Deactivation of immune system

The introduction of HAART has altered the course of HIV-1 infection. A rapid decrease in the viral load (VL) and a significant decrease of cellular and soluble markers of immune activation are followed by increase in CD4+ T-cell counts [129, 144]. However, successful suppression of the VL is not immediately followed by normalisation of T-cell levels that takes many years and is not always achieved [145, 146]. This observation implies that activation of the immune system, not direct viral effect followed by homeostatic compensatory mechanism is a major cause of T-cell

death. In any case, the effect of HAART strongly supports the role played by the virus in the cause of abnormal activation of immune system. Suppressing the virus and hampering of the immune activation not only restore number and function of the T-cells but also has the important impact on the other immune cells, such as CD8+ T-cells, B-cells and polymorphonuclear cells [112]. However, the deactivation of immune system may not be the ''golden approach'' in the fight with HIV-1, as the proper level of immune activation is essential for its function. This could also be illustrated by the rapid decline in the HIV-specific CD8+ T-cells counts during the first month of an efficient antiretroviral therapy [144, 147]. Furthermore, clinical trials with the use of anti-inflammatory and/or immunosuppressive drugs have not shown a clear picture of advantage in delaying disease progression [148, 149]. Hydroxyurea, prednisone and mycophenolic acid have been tested for these purposes, showing limited benefit (or even harm) that dampened the enthusiasm for this approach [150]. Interesting results have been obtained with another immunosuppressive agent, Cyclosporin A (CsA). This drug acts on lymphocytes and prevents T-cell activation, reduces cytokine production and adhesion molecule expression [151]. Additionally interacting with cyclophiline, CsA has been shown to exert direct anti-HIV effect [152]. A study involving treatment of PHI patients with HAART and 8-weeks CsA regime showed positive effect of this combination on CD4+ T-cell counts and function [153]. This effect was pronounced and sustained for >1 year as the follow up study reported [154]. However this beneficial effect of CsA was not confirmed in a clinical trial involving chronically infected individuals [155].

There are still gaps in the basic knowledge of the concomitant regulation of viral replication and immune activation. A better understanding of how HIV-1 induced immune activation leads to CD4+ T-cell depletion may provide new targets for immune-based interventions that could be used in addition to HAART to slow disease progression [156].

High mobility group box protein 1 (HMGB1)

HMGB1 is a member of the family of high mobility group (HMG) proteins (A, B, N) (http://www.informatics.jax.org/mgihome/nomen/genefamilies/hmgfamily.shtml). They were first described in the 1970s, as a group of non-histone DNA binding proteins and named because of their rapid mobility on polyacrylamide gels under electrophoresis [157]. HMGB1 is the most ubiquitous of the high mobility box (HMGB) sub-family, being constitutively expressed in all nucleated cells, though in variable levels [158, 159]. HMGB2 and HMGB3 are primarily expressed during embryogenesis, however expression of HMGB2 in the testis and lymphoid tissue in adult mice has also been reported [160-162].

HMGB1- the intracellular protein

HMGB1 consists of 215 amino acids with the gene localisation on chromosome 13q12 [163]. It is highly conserved through evolution with 99% homology between rodents and humans. Structurally, HMGB1 is organized into two DNA-binding domains (named A-box and B-box) and a negative charged C-terminus (Figure 4) [164]. The two DNA-binding domains have an α-helix conformation and attach to minor grooves of linear DNA, which results in DNA bending. This is considered as an allosteric transition of DNA, which can promote the binding of other proteins. HMGB1 implies to regulate the transcription of several genes through two potential mechanisms [159]. The first mechanism is the ability of HMGB1 to interact directly with nucleosomes (by loosening the wrapped DNA) and enhance accessibility to chromatin-remodelling complexes and possibly to transcription factors. The second mechanism is exerted by establishing protein-protein interactions. Thus, HMGB1 binds various transcription factors, including p53, recombination activating gene 1 / 2 (RAG 1 / 2) proteins, several NF-κβ subunits and all steroid (class I) receptors [165, 166]. These interactions enhance/regulate transcriptional activation. HMGB1 overexpression, observed in many tumour cells, accelerates cell-cycle progression probably through its ability to promote access to some transcriptional complexes [167]. The nuclear functions of HMGB1 are critical for survival but not for foetal development, as HMGB1-knockout mice are born alive although they die within 24h due to hypoglycaemia [168]. Cell lines obtained from these animals grow normally, but the activation of the gene expression by different factors, including the glucocorticoid receptors, is impaired.

Figure 4. Schematic structure of HMGB1 protein. The two nuclear localisation sequences (NLS) are positioned according to the primary amino acid sequences of human HMGB1. Modified from [164]

HMGB1- the extracellular protein

The year 1999 publication of *Wang et al* [169] has attracted intensive interest in the properties of HMGB1. The authors have described that this intracellular protein could be actively released after stimulation of macrophages/monocytes and functions as a late mediator of lethal endotoxemia and sepsis. However, prior to Wangs article other groups have proposed that HMGB1 could function as an extracellular factor, in particular with regard to cell differentiation and motility [170, 171].

Release of HMGB1

Active secretion of HMGB1 has been described in macrophages/monocytes, dendritic cells, pituicytes, NK and endothelial cells, following the stimulation with exogenous bacterial products such as endotoxines or proinflammatory cytokines (IL-1β, INF-γ, TNF-α) [164]. The mechanism of active HMGB1 secretion is not yet thoroughly understood. It is established that HMGB1, like IL-1β and fibroblast growth factors, is lacking a classical leader sequence and can not be released by classical endoplasmic reticulum-Golgi mediated pathway. Instead, activated macrophages/monocytes acetylate HMGB1 at potential nuclear localization sequences, leading to its cytoplasmic translocation and subsequent release into the extracellular milieu through secretory vesicles [172]. The acetylation step seems to control active HMGB1 release, at least in myeloid cells [173]. Scaffidi et al [174] reported an alternative mechanism for HMGB1 release, demonstrating that necrotic or damaged cells release bioactive HMGB1. The underacetylation of chromatin that precedes formation of apoptotic bodies causes tightly binding of HMGB1 in apoptotic cells that prevents HMGB1 release. Even secondary necrosis or partial autolysis was not affecting the HMGB1 release. Moreover, necrotic cells lacking the HMGB1 were not able to induce inflammation as compared to normal cells. This distinction in HMGB1 liberation between the models of cell death could explain the presence of inflammation associated with necrosis and its absence during apoptosis [166, 175].

Alarmin

Recently a new term ''alarmin'' was proposed to describe endogenous molecules that could signal tissue and cell damage to immune cells [176, 177].

They have been applied characteristics such as:

1) Rapid release following the non-programmed cell death, but lack of release during apoptosis
2) Alternative pathway of active secretion by cells of immune system
3) Stimulation of innate and adaptive immunity
4) Role in the tissue repair and regeneration

All above criteria can be met by HMGB1. This molecule activates immune cells and promotes the release of proinflammatory cytokines as well has chemotactic activity [164]. Andersson et al [178] have reported secretion of TNF-α, IL-1α, IL-1β, IL-1RA, MIP-1α, MIP-1β, IL-6 and IL-8 by macrophages stimulated with HMGB1. A similar pattern of cytokine release has been described in other cells [179]. The chemotactic properties of HMGB1 have been described on many cells including monocytes, macrophages, dendritic cells, enterocytes, endothelial and smooth muscle cells [166, 180]. Moreover, HMGB1 has been attributed proangiogenic activity [181]. Neurons respond to the protein by extending neurites, the process that also involves cytoskeleton remodelling and resembles chemotaxis [182]. HMGB1 stimulates maturation of dendritic cells which implicates a role in switch from innate to adaptive immune system [183, 184]. In addition, HMGB1 can recruit stem cells and its involvement in tissue regeneration after myocardial infarction has been reported [185].

Extracellular HMGB1 binds to a cellular receptor for advanced glycation endproducts (RAGE) in a concentration-dependent manner [186]. RAGE is a transmembrane protein that belongs to the immunoglobulin superfamily, and acts as a receptor for the diverse ligands including the amyloid peptide and members of S100/calgranulin family of inflammatory mediating peptides [187]. It is expressed at low levels in normal tissues and in the vasculature. Increased levels of RAGE expression has been found in diabetes, Alzheimer's disease and atherosclerosis [188]. Stimulation of RAGE results in the activation of multiple intracellular signalling pathways including the mitogen activated protein kinases, Rho GTPases Rac and CDC42, as well as NF-κβ translocation [179]. These signalling pathways allow the transcription of pro-inflammatory genes, cytokine production finally inducing inflammatory response. Recent structural/functional studies have localized the cytokine functional motif (amino acids 106-123) in the ''B-box'', but the RAGE-binding motif in C-terminus (Figure 4), suggesting a potential involvement of other receptors in HMGB1 induced activation [164]. Accumulating evidence indicate that in addition to RAGE, toll like receptors (TLR-2 and TLR-4) and syndecan-1, also mediate the cytokine activity of HMGB1 [182, 189].

Role of HMGB1 in inflammation

Several experimental models have been used to demonstrate that HMGB1 plays a central role in acute and chronic inflammation, including endotoxemia, septicaemia, rheumatitis and acute respiratory distress syndrome (ARDS) [164]. Thus, high systemic levels of HMGB1 in animals and correlation to symptoms as well survival have been shown [190]. Injection of recombinant HMGB1 into mice causes the development of clinical signs of sepsis and multiple organ failure [191]. In addition, passive immunization of anti-HMGB1 neutralizing antibodies increased the survival rate in the mice sepsis model [192]. In humans, increased systemic HMGB1 levels have been reported in different organ disorders and diseases characterized by inflammatory responses, including sepsis, pancreatitis, disseminated intravascular coagulopathy, rheumatoid arthritis (RA) and ARDS [169, 193-196]. However, the correlation between survival rate, clinical symptoms, parameters of immune

activation and levels of HMGB1 vary significantly between studies most probably due to differences in the methodology and treatment-related interventions [197]. Neither animal, nor human studies have answered the questions about the origin and biological importance of circulating HMGB1 [179]. In the recent publication *Urbonavicute et al.* [198] reported the presence of anti-HMGB1 antibodies in plasma/sera not only in individuals with autoimmune diseases but also in healthy subjects. The authors have also described the occurrence of elevated HMGB1 levels among the latter group. These findings are not theoretically astonishing, but presume complex regulation of HMGB1 function *in vivo*. Their importance, both in the relation to methodology of previous reports and clinics, remains to be elucidated.

The plethora of functions governed by HMGB1, especially the potent proinflammatory activity, have attracted a lot of interest and resulted in a magnitude of publications after the re-discovery of HMGB1. During the last 6 years the view on the HMGB1 protein has fluctuated from being a danger signal and a potent proinflammatory cytokine *per se* to rather being an immunomodulatory factor [176, 199]. The role of HMGB1 as an important mediator in cell death and modulation of immune responses is not questioned, however future research would probably place this interesting molecule in a more correct position [177]. Even though, HMGB1 is a potential target for immunotherapy [192].

Role of HMGB1 in viral infections

The function of HMGB1 depends on its localisation [200], which also attributes to viral infections. The nuclear localization and function of HMGB1 imply its possible influence on the viral transcription in infected cells. HMGB1 has been shown to stimulate transcription from viral promotors, such as the adenovirus major late promotor (MLTF) and the SV40 late promoter [201]. Gamma-2 herpesviruses, including Kaposi sarcoma-associated virus (KSHV) and murine gamma herpesvirus 68 (MHV-68), exploit HMGB1 to facilitate binding of their RTA (replication and transcription activator) protein to different promoters [202]. Moreover, MHV-68 replication, in addition to its gene expression was reduced in HMGB1 -/- cells. Phosphoprotein P of Borna disease virus (BDV) binds A-box subunit of HMGB1 and represses the transcriptional activity of p53 in infected cells [203]. This strategy of P protein may be involved in noncytolytic replication and persistent infection of BDV in CNS cells. As different cell types express diverse amounts of HMGB1 its intracellular interaction might be cell specific [159].

Viral infections trigger inflammatory responses that involve both innate and adaptive immunity [204]. This is reflected by increased levels of local and systemic immune activation. If the inflammatory response is appropriate the infection will be cleared, however the overproduction of cytokines and chemokines may become harmful to the host and cause organ injury [205]. Many viruses are directly cytopathic causing cell necrosis, release of HMGB1 and inflammation. This has been proven for West-Nile (WN) virus belonging to the *Flaviviridae* family, which at a high infectious dose induces necrotic cell death caused by profuse budding of WN progeny virus particles [206]. Similar observations have been made for *in vitro* infection with Salmon

anaemia virus [207]. Death of CD4+ T-cells is a hallmark of HIV-1 infection [15], even if the relative contribution of apoptosis/necrosis to this process is still a matter of discussion [208]. The direct cytophatic effect HIV-1 seems to be essential in the primary and late phases of the infection [81]. Unpublished data from our laboratory (P. Nowak et al, Manuscript in preparation) reveal that HIV-1 infection of T-cell lines and PBMCs results in cytopathic effects and subsequent HMGB1 release (Figure 5).

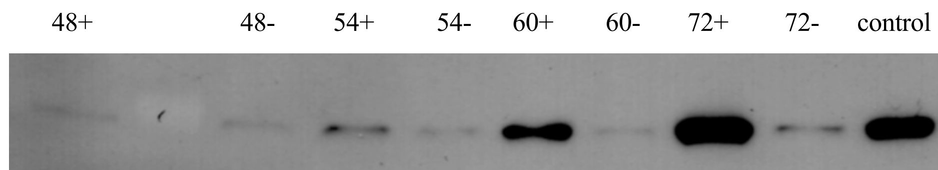

Figure 5. Kinetics of HMGB1 release from MT4 cells infected with HIV-1 virus. Western-blot technique was applied to detect the HMGB1 protein in supernatants of HIV-1 infected (+) and uninfected (-) cells at 48, 54, 60, 72 hours after infection. Cytopathic effect in the HIV-1 infected wells was present at 60h. Supernatants from necrotic MT4 cells (obtained by three rapid freezing and thawing cycles) served as a control.

Besides the contribution to immune activation of HMGB1 caused by the passive release, an active secretion could be assumed based on several observations [205]. In viral infections increased local and systemic levels of several pro-inflammatory cytokines have been documented [209, 210]. An upregulation of the HMGB1 expression in a mice model of experimental hepatitis has been described [211]. Moreover, treatment of CTL-induced liver disease in HBV transgenic mice with inhibitors of extracellular HMGB1 significantly decreased the intrahepatic recruitment of inflammatory cells and diminished the severity of liver disease [212]. These findings implicate that HMGB1 released both in active and passive manner could initiate an inflammatory response and contribute to viral pathogenesis (Figure 6).

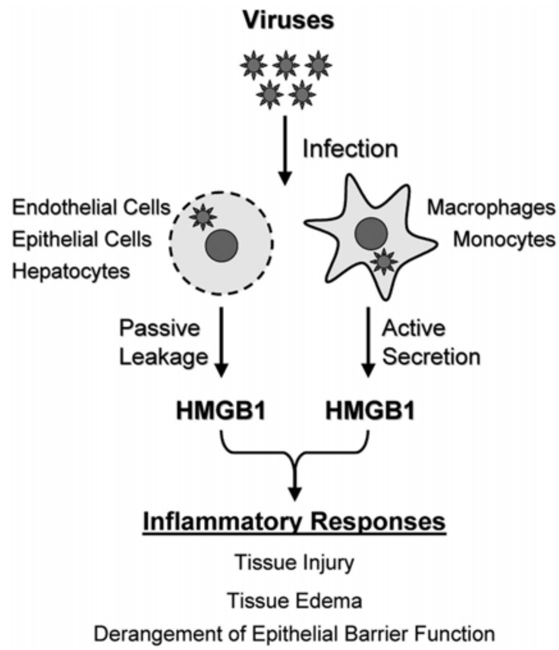

Figure 6. Hypothetical role of HMGB1 in the pathogenesis of viral infectious diseases. Adapted from [205].

Aims of the study:

General aim

- To investigate the role exerted by immune activation in HIV-1 infection, as well as to characterise the contribution of HMGB1.

Specific aims

- To analyse the impact of a heavy immunosuppressive regime on the HIV-1 kinetics and sequence variability after liver transplantation in patients with anti-HIV-1 treatment (Paper I)
- To determine the effect of intracellular HMGB1 overexpression on HIV-1 transcription (Paper II)
- To investigate the effect of extracellular HMGB1 on HIV-1 replication in acute and chronic models of HIV-1 infection (Paper III)
- To determine systemic levels of HMGB1 in HIV-1 infected patients (Paper V)
- To investigate the pattern of immune activation by studying cytokines and chemokines during primary HIV-1 infection and after analytical treatment interruption during chronic HIV-1 infection (Paper IV)

Material and Methods

For details about the Material and Methods used in this thesis, see respective paper.

Ethical clearance

The studies included in thesis were performed in the spirit of Helsinki agreement, after approval from the Institutional Review Boards and Ethical Committees at each participating site. All subjects included in the study gave their signed informed consent prior to study enrolment.

Statistical analysis

For details about the statistical analysis used in this thesis, see respective paper.

Results and Discussion

Background- role of immune activation in HIV-1 infection

The central question that I have tried to address in my thesis is; what is the role of immune activation in HIV-1 pathogenesis? Being a physician taking care of patients and with the knowledge that the activation of the immune system in the end cause severe harm to the infected individual, I chose to perform both *in vivo* and *in vitro* studies. In order to analyse whether an advanced immunodeficiency induced by chemotherapy could affect the course of the HIV-1 infection in patients, my studies started with the characterisation of the HIV-1 disease in an extraordinary group of patients - those who underwent orthotopic liver transplantation (OLT) and were treated with both immunosuppressive and antiretroviral regimes. In addition, I also studied another unique group of patients with primary HIV-1 infection (PHI), the largest in the world ever studied, in whom ART where initiated very early and who also participated in the first study ever performed with therapeutic vaccination in such patients – the Quest study. Studying these patients I managed to characterise the very early immune activation events and compare the events occurring after analytical treatment interruption (ATI) in the same patients. This was done by analysing the kinetics of 13 soluble markers of immune activation, both cytokines and chemokines, in samples obtained both during PHI and after ATI. In the latter part of my work, I characterized the involvement of the newly re-discovered HMGB1 protein in the interaction with HIV-1 both in *in vitro* and *in vivo* settings.

Paper I: Follow up of antiretroviral treatment in liver transplant recipients with primary and chronic HIV-1 infection

Background for the study

Introduction of ART has dramatically improved the prognosis of HIV-1 infected patients and has changed the picture of the disease course [95]. Progression to end-stage liver failure now frequently occurs in subjects co-infected with hepatitis C virus (HCV) [213]. This has raised again the issue of organ transplantation in such patients, however due to experience from pre-ART era, HIV-1 infection was considered a contraindication to organ transplantation in many transplant centres [214]. There have been several concerns:

1) Immunosuppressive treatment would lead to an increased viral replication and disease progression [215].
2) Drug interactions between the components of ART: protease inhibitors, nonnucleoside analogues and immunosuppressive compounds [216]. These drugs utilize similar cellular transport systems and the same metabolizing enzymes of the cytochrome P450. Concomitant usage of these agents would require careful monitoring.
3) The limited access to liver transplants made transplantation ethically questionable as long as the prognosis of the HIV-1 infection itself was bad [217].

Nevertheless, the increased need for transplants, accumulating information about the negative role of immune activation in HIV-1 pathogenesis as well as increased possibilities to perform therapeutic drug monitoring of the immunosuppressive and antiretroviral drugs resulted in the re-evaluation of transplant lists criteria. At Karolinska University Hospital, one of the first liver transplantations of an HIV-1 infected patient ever done was performed in 1998 and a further four patients were transplanted during the following years. In view of aspects discussed above, we decided to study how intensive immunosuppression in combination with potent anti-HIV therapy could influence the viral and immunological events in four patients after OLT.

Absence of increased HIV-1 replication and of increased viral evolution in patients under immunosuppressive treatment

Three HIV-1 infected patients (patients 1, 2, 3) with liver cirrhosis due to chronic HCV infection and one patient (patient P) with primary biliary cirrhosis (PBC) were followed up 3, 9, 14 and 36 months after OLT. All subjects were treated with both antiretroviral and immunosuppressive drugs. The PBC patient was ‘’exceptional’’ as he developed PHI at the time of transplantation. We investigated the effect of the combined therapy on HIV-1 viral load (VL), viral diversity and divergence as well CD4+ and CD8+ T-cell counts.

Our data strongly support the pivotal role of immune activation in the HIV-1 pathogenesis. A detailed analysis of the viral events in our four patients showed that HIV-1 replication was efficiently inhibited by the potent ART without any significant support provided by the HIV-1 specific adaptive immunity. Thus, in the chronically infected patients, VL was undetectable (patients 1 and 3) or declined (patient 2) during post OLT follow up. The kinetics of VL in patient P (Figure 3, paper I) followed initially the pattern generally observed in PHI patients [82]. The peak of VL occurred 1 week after onset of symptoms and decreased thereafter, before the initiation of ART but under the immunosuppressive regime. It is thus unlikely that this initial viral decrease was due to effects of the adaptive immunity. We suggest that this decline was more likely due to an innate immune response or rather, as implied from the data of Paper IV, a decrease of immune activation and in consequence a lack of target cells for the virus. In opposite to the third phase of slower viral decline usually seen in PHI, the viremia increased in patient P. As similar VL kinetics were observed in CD8+ T-cell depleted SIV infected monkeys [215], the role of CTLs in controlling these early viral events could be considered [218]. In patient P, a decreased immune activation was not only restricting the access to target cells but also dysregulating appropriate immune response to the pathogen. The efficacy of ART was further illustrated by the gradual plasma HIV-1 RNA and cellular HIV-1 DNA decrease after treatment initiation.

Moreover, the restriction of the viral evolution in PHI and chronic HIV-1 infected patients, measured as viral diversity and divergence (Table 1, paper I), was similar to data reported from corresponding patient groups who did not received immunosuppression due to transplants [76, 219]. We did not observe a deterioration

of CD4+ T-cell counts in our patient group. On the contrary a stable increase was seen in 3 out of 4 individuals.
In conclusion, our data show that suppression of immune system in HIV-1 infected patients under effective HAART does not eventually lead to increase of viral replication and to rapid disease progression.

Papers II, III and V- The role of HMGB1 in HIV-1 infection.

Background for the study

We intended to characterise the function of the HMGB1 in HIV-1 infection by studying different aspects of the virus and protein interaction *in vitro* and by analysing plasma HMGB1 levels in HIV-1 infected patients.

HMGB1 is a remarkable molecule. Although, very abundant in all nucleated cells, the clear-cut intracellular function is still the matter of debate [159]. Based on sequence homologies, the DSP-1 protein in Drosophila has been suggested to be the equivalent of vertebrate HMGB1 [220]. DSP-1 can convert members of Rel family of transcription factors, NF-κβ and the Drosophila protein Dorsal, from being transcriptional activators to repressors [221]. Similar to DSP1, the HMGB1 can interact with Dorsal and Rel proteins: p50 and p65 [165]. Thus, cells express different amounts of HMGB1, which could be attributed to a cell specific modulation of transcription [159]. The data presenting HMGB1 interactions with viral transcription factors with stimulatory/ inhibitory consequences have implicated its potential role during viral infections [201-203]. Moreover, the presence of NF-κβ binding sites, as well as cell specific differences in HIV-1 pathogenesis, has encouraged us to test the possibility of HMGB1 involvement in the intracellular modulation of HIV-1 transcription.

Reports showing that HMGB1 could be released to the extracellular environment and there act as a proinflammatory cytokine [190], brought to us another important facet of HMGB1 and HIV-1 interactions. An obvious question was if exogenous HMGB1 could act on HIV-1 infected cells and stimulate viral replication. Several proinflammatory cytokines can stimulate viral production from infected cells and contribute *in vivo* both to an increase of VL as well as immune activation and viral spreading [133]. The biology of HMGB1 could add an additional mechanism to immune activation in HIV-1 infection. Necrotic cell death present in HIV-1 infection [222] could liberate HMGB1 [174] and add an endogenous factor that will endeavour a vicious cycle of immune activation and viral replication. Elevated levels of HMGB1 have been shown in several conditions to be associated with acute and chronic inflammation/immune activation [190]. Activation of the immune system is a hallmark of HIV-1 infection correlating to disease progression [108]. The potential *in vivo* link between systemic levels of HMGB1, virological and immunological parameters of HIV-1 disease was therefore worth testing.

Intracellular HMGB1 represses HIV-1 replication in epithelial cells (Paper II)

To test the effects of intracellular HMGB1 on HIV-1 replication, the full length HIV-1 infectious clone (pNL4-3) was cotransfected in the absence or presence of various concentrations of HMGB1 sense, HMGB1 antisense, or the empty pcDNA vector, into HeLa, Jurkat and Thp-1 cells. In HeLa cells, we observed inhibition of HIV p24 *gag* production from pNL4-3 in the presence of HMGB1 sense plasmid, at 24 hours post transfection. This inhibitory effect of HMGB1 was dose dependent with a maximum of five fold inhibition at ≥ 5μg of HMGB1 sense plasmid (Figure 1, Paper II). Moreover, this repressive effect was seen independently of hGH normalization. No inhibition of viral replication was observed in the presence of empty pcDNA or antisense HMGB1 plasmid indicating that the inhibitory effect was induced by ectopic production of HMGB1. In Jurkat cells, we could not observe any modulatory effects on viral replication with any of the transfected HMGB1 plasmids. This may suggest an inhibitory effect of HMGB1 through NF-κβ since there is no activated NF-κβ in the nucleus of Jurkat cells, in contrast to HeLa cells. Analysis of cell lysates, revealed that Thp1 cells carried more HMGB1 than the epithelial and T-cells. Therefore, transfection with HMGB1 sense plasmid did not have any effect on pNL4-3 replication. On the opposite, a two fold upregulation of viral replication was observed in the presence of 5μg antisense HMGB1 plasmid at ≥ 24 hours post transfection (Figure 6, Paper II).

Intracellular HMGB1 represses HIV-1 LTR mediated transcription in HeLa cells (Paper II).

To investigate whether the HMGB1 repressive effect was at the level of LTR-directed transcription, a CAT reporter gene construct containing HIV-1 LTR derived from subtype B virus (pNLCATB) was cotransfected in the presence or absence of sense and antisense HMGB1 plasmids. Ectopic overexpression of HMGB1 inhibited transcription directed from PNLCATB HIV-1 LTR (Figure 2, Paper II). Analogous effect was present when HeLa Tat cells were used as target for transfections. This inhibitory effect of HMGB1 was LTR specific in the sense that HMGB1 in contrast stimulated the transcription from SV40 promoter/enhancer. Additionally, HMGB1 had no substantial effect on CMV promoter. In accordance with these findings, HMGB1 has been shown to stimulate transcription from adenovirus major late promoter and the SV40 late promoter [201].

We further investigated a possible functional interaction between HMGB1 and NF-κβ in context of HIV-1 LTR. By deletion of the extra NF-κβ site (NF-κβI) present in the LTR of subtype C viruses, we could show a higher inhibitory activity of HMGB1 on the NF-κβI mutant subtype C LTR compared to the WT subtype C LTR. The interaction of HMGB1 with NF-κβ alone cannot be the only explanation for HMGB1 repression of HIV-1 gene expression because several numbers of NF-κβ sites are also present in the SV40 promoter/enhancer in which, in contrast to HIV-1 LTR is stimulated by HMGB1. It is generally acknowledged that transcription factors bind to each other in a three-dimensional jigsaw puzzle creating a multiprotein/DNA complex [159]. Thus, it can be speculated that a ternary complex forms between HMGB1, NF-κβ and another, yet unidentified repressor(s) which directly or

indirectly interact with specific element within LTR. Similar regulation of HIV-1 LTR transcription for other proteins interacting with NF-κβ site has been reported [31, 223-225].

Extracellular HMGB1 upregulates HIV-1 replication in latently infected monocytic cells (Paper III)

To investigate the role of extracellular HMGB1 on HIV-1 replication, we added recombinant HMGB1 to the U1 cell line, which is accepted as a model of latent HIV-1 infection in monocytic cells [226]. We could observe three fold increase of viral replication (measured as RT upregulation in supernatants) in HMGB1 stimulated cells as compared to controls (Figure 1, paper III). This effect was dose dependent and specifically linked to the protein as Polymyxin B was used to inhibit endotoxin contamination. Additionally, presumed amounts of LPS tested in 100 fold higher concentrations did not have stimulatory effect on HIV-1 replication in U1 cells. Experiments with dexamethasone inhibition of the stimulatory effect suggested that the downstream pathway of HMGB1 stimulation in U1 cells involves utilization of NF-κβ complex. HMGB1 did not have any effect on stimulation of viral replication in chronically infected T cells (ACH-2). This observation is in line with the initially suggested absence of stimulatory effect of HMGB1 on lymphoid cells [178]. Our findings implicate that HMGB1 could act as a proinflammatory cytokine on latently infected cells of monocytic origin. Recently published work by Thierry et al [227] has confirmed our findings in the U1 cell model. Moreover, their experiments with protein synthesis inhibitor cyclohexamide, revealed that HMGB1 acts directly on viral transcription, without additional stimulation of proinflammatoric mediators. The authors report also divergent finding concerning the effect of HMGB1 on HIV-1 replication in ACH-2 cells. One possible explanation of this apparent discrepancy might be different basal levels of viral expression as well different read-outs used in our experiments. Thus, this finding is surprising, as there is no information on direct effect of exogenous HMGB1 on function of T-cells. However, T-cells express RAGE receptor and the possibility of such interaction can not be dismissed.

Extracellular HMGB1 represses HIV-1 replication in acutely infected macrophages (Paper III)

Addition of HMGB1 to acutely infected (7 days) monocytes derived macrophages (MDM) resulted in inhibition of viral replication present already at 24 hours post HMGB1 stimulation (Table 1, paper III). This effect was similar in MDM cultured in adherent and non-adherent conditions. Analysis of the culture supernatants revealed HMGB1 induced release of RANTES, MIP-1α, and MIP-1β from MDM cells. These cytokines have been attributed the inhibitory effect, not only during HIV-1 entry, but also on viral replication in macrophages [228]. Thus, it is very likely that their anti-HIV-1 effect was exerted in the HMGB1 stimulated HIV-1 infected MDMs. Induction of MIP-1α and MIP-1β from monocytes by HMGB1 stimulation has been previously described [178]. Our data indicate that RANTES should also be included in this pattern. This double mode of action; suppressive in the acute model and stimulatory in the chronic model of HIV-1 infection is not unusual for the effects of

cytokines on HIV-1 replication. Similar properties have been documented for e.g. TNF-α, INF-γ and endotoxin [139, 228-231].

Increased plasma levels of HMGB1 in HIV-1 infected patients (Paper V)

In Paper V, we determined the levels of HMGB1 in plasma from 14 healthy HIV-1 negative volunteers and 43 treatment-naïve HIV-1 infected individuals using an ELISA system. Three patient-categories were defined based on immunological and virological parameters, as well as presence of opportunistic conditions at the time of blood sampling (Table 2).

Table 2. Characteristics of HIV-1 infected patients studied in Paper V.

	Group A (n= 14) Patients with CD4+ T-cells >600/ul	*Group B (n= 13) Patients with CD4+ T-cells <400/ul*	*Group C (n=16) Patients with CD4+ T-cells ≤300/ul and opportunistic conditions**
Female/Male, n	6/8	4/9	8/8
Age (years)[a]	39 (27-66)	36 (24-53)	39 (23-62)
Transmission			
Homosexual, n	1	1	2
Heterosexual, n	11	7	12
IVDU[b], n	2	5	2
HCV infection, n	2	5	3
CD4+ T-cell count (cells/μl)[a]	950 (655-1 519)	246 (58-374)	85 (8-300)
CD8+ T-cell count (cells/μl)[a]	1165 (429-3050)	815 (153-1498)	667 (88-1864)
HIV-1 RNA load (copies/ml)[a]	350 (19-1 900)	347 000 (20 000-10^6)	541 000 (8 900-10^6)

[a]median (range) [b]IVDU, Intravenous drug users

*Opportunistic conditions in 16 HIV-1 infected patients (group C): *Patient (P) 1*: Pneumonia (bacterial); septicaemia; *P2*: Tuberculosis (TB) (disseminated); *P3*: B-cells lymphoma; *P4*: Oropharyngeal candidiasis; TB (lymph node); *P5:* Aspergillosis; TB (lymph node); *P6: Penumocystis jiroveci* pneumonia (PCP); *P7:* TB (pulmonary); Oesophageal candidiasis; Toxoplasmosis; *P8:* Wasting syndrome; Oropharyngeal candidiasis; *P9:* Pneumonia (bacterial);*P10*: Oropharyngeal candidiasis; *P11:* TB (lymph node); *P12:* Oesophageal candidiasis; Kaposi's sarcoma; Wasting syndrome; Neurosyfilis; *P12: Mycobacterium avium* complex infection (disseminated); Oesophageal candidiasis; Toxoplasmosis; PCP; *P13-16:* TB (pulmonary).

We found higher plasma levels of HMGB1 in the HIV-1 infected patients, as compared to HIV-negative healthy controls ($p<0.001$). Additionally, HIV-1 infected patients with opportunistic conditions had higher HMGB1 levels as compared to asymptomatic patients with preserved ($p<0.05$) or deteriorated immune system ($p<0.05$) (Figure 1, Paper V). There was no significant difference in HMGB1 levels between the two latter groups. No difference was observed in HMGB1 levels within the symptomatic patients with regard to specific opportunistic conditions. Additionally, no statistically significant correlation was found between the plasma HMGB1 levels and sex, age, viral load, CD4+ or CD8+ T-cell counts, respectively. Our results thus show that HMGB1 can be detected in the circulation of chronically infected HIV-1 individuals with the highest concentrations in patients with clinical complications. The data could support the view that a profound activation of immune system is related to the high HMGB1 levels. In addition, cytopathic effects of HIV-1 may contribute to the enhanced HMGB1 levels as suggest our unpublished data derived from *in vitro* system (Figure 5).

The levels of HMGB1 found in HIV-1 infected patients were comparable to those in patients with disseminated intravascular coagulation or with acute pancreatitis [194, 196]. The clinical relevance of the high systemic HMGB1 levels remains to be elucidated. The work showing existence of anti-HMGB1 antibodies not only in patients with autoimmune diseases but also in healthy individuals [198], implicate that the regulation of HMGB1 function is very complex [177].

Paper IV. Kinetics of plasma cytokines and chemokines during PHI and after analytical treatment interruption.

Rationale for the study

Although systemic immune activation is an important pathogenic factor in chronic HIV-1 disease, its role in primary HIV-1 infection (PHI) is poorly defined [108]. However, the events occurring during this early phase of HIV-1 infection are critical for disease prognosis [81, 110]. Thus, a functional impairment of the cellular response early in the course of infection has provided a rationale for the initiation of ART during acute infection to preserve HIV-1 specific T cell responses and decrease immune activation [232]. Cytokine imbalances play a key role in HIV-1 pathogenesis through the upregulation of viral replication and immune activation [136]. There is a lack of comprehensive data about the kinetics of these soluble factors during PHI. A better understanding of their contribution to the immune activation during PHI could help develop novel treatment strategies.

In this study, we analysed the pattern of immune activation by measuring the kinetics of 13 cytokines and chemokines in 22 patients during PHI and after analytical treatment interruption (ATI). Patients enrolled in this sub-study were participants of the QUEST study, the first larger controlled treatment trial in acute HIV-1 infection [233]. It was also the first clinical trial of therapeutic vaccination [234]. The Quest cohort was very unique as the patients were enrolled very early after exposure to HIV-1. The first sample was drawn before a complete antibody pattern was

developed (as identified on the Western blot), corresponding to two-three weeks after HIV-1 infection. The selection of the patients, in our substudy, was based on the virological outcome at the end of the study as well as availability of plasma samples. The individuals were divided into two groups: further referred as responders (R) who had VL levels <1000 HIV-1 RNA copies/ml and non-responders (NR) who had VL >9000 copies/ml at 6 months after treatment interruption [235]. The ART was initiated very early during the PHI and lasted at least for 96 weeks for all subjects.

Intensive immune activation during PHI is associated with worse virological outcome after analytical treatment interruption

During PHI, the NR had significantly higher levels of HIV-1 RNA, IFN-γ, TNF-α, IL-1β, IL-10 and Eotaxin than the R ($p \leq 0.05$). Furthermore, a positive correlation was found between VL and IFN-α, TNF-α, IL-1β, MIP-1α and MIP-1β, respectively. On the contrary, R had significantly higher levels of IFN-γ, MIP-1β and MCP-1 than NR after ATI, while NR had higher levels of HIV-1 RNA, IL-15 and Eotaxin (Figure 7).Comparison between the cytokine/chemokine concentrations during the highest viraemia point of both phases showed significantly higher levels of IFN-γ, TNF-α, IL-10 and chemokines (with exception of MCP-1) during PHI than post-ATI.

The data describe different cytokine profiles during PHI and post ATI. All subjects showed an intense state of immune activation during PHI, the degree of which correlated with the VL, and was higher in the non-responders. Thus, subjects who had lower levels of VL and of all soluble factors at PHI (with the exception of MCP-1) also had lower VL after ART cessation. It implicates that the problem during PHI is not a lack of certain immune mediators as seen in other infections [236-238]. The profound activation of immune system is likely to be caused primarily by the extensive viral replication during PHI [81]. The question remains to be answered if and which genetic factors [239] determine the lower degree of immune activation in R.

This lower degree of immune activation might cause better preservation of the innate and adaptive immunity in those individuals. Betts et al has recently [240] suggested that presence of polyfunctional CD8+ T-cells characterized by strong cytokine (INF-γ, TNF-α, IL-2) and chemokine (MIP-1β) production is negatively correlated with disease progression. Our data from the plasma compartment are in line with this hypothesis.

In conclusion, our work adds some important information in terms of the pattern of cytokine and chemokine production during early HIV-1 infection and after cessation of ART. We have shown that, the degree of immune activation was highest during PHI and associated with VL levels. We also showed that lower levels of viral replication and immune activation during PHI are associated with improved long-term virological outcome. Further investigations are required to clarify the role of ART in the change of cytokine pattern observed after treatment interruption and its potential impact in the long-term virological outcome.

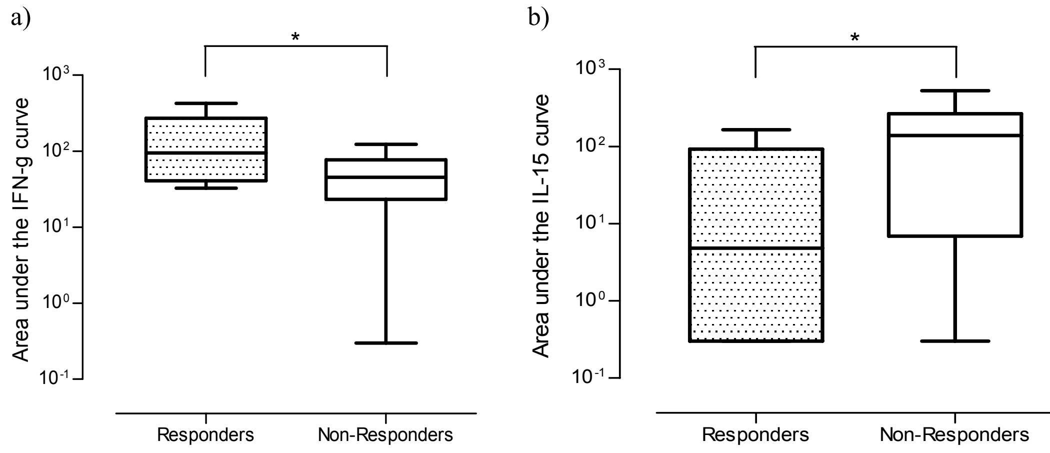

Figure 7. The graph show the comparison between the area under the a) INF-γ curve and b) IL-15 curve during the post ART phase in responder and non-responder group. * $P<0.05$

Concluding remarks

1) Potent antiretroviral therapy without any support from concomitant HIV-1 specific adaptive immunity was sufficient to efficiently control HIV-1 replication in patients after OLT. Chemotherapy induced suppression of the immune system under successful ART did not lead to an increase of viral sequence evolution or a more rapid disease progression.

2) Endogenous HMGB1 expression represses HIV-1 LTR-directed transcription in a promoter and cell specific manner. Constitutive higher expression of HMGB1 in monocytes/macrophages could be responsible for slow mode of HIV-1 replication in these cells.

3) Extracellular HMGB1 induces HIV-1 replication in latently infected monocytic cells but not T-cells. The opposite effect of exogenous HMGB1 stimulation is seen in acute infected primary macrophages. The inhibition of HIV-1 replication in these cells could be explained by induction of β-chemokines production by HMGB1.

4) Plasma levels of HMGB1 are elevated in HIV-1 infected patients, with the highest concentration in patients with clinical complications. HMGB1 is likely to contribute to the activation of the immune system in HIV-1 infection.

5) High levels of immune activation during PHI are associated with high HIV-1 RNA levels rather than better control of the viral replication. Additionally, the degree of immune activation during PHI may determine the functionality of immune system as well as the virological outcome post-analytical treatment interruption. This interaction does not seem to be affected by early antiretroviral therapy.

Populärvetenskaplig sammanfattning

En abnorm aktivering av immunsystemet är central för sjukdomsutvecklingen vid HIV-infektion och AIDS. Denna leder till ett oproportionellt kraftigt och missriktat immunsvar och till att specifika celler i immunförsvaret dör, vilka också angrips av HIV. Hypotesen är att HIV leder till en kronisk inflammation med ett inadekvat immunsvar som förklarar det avvikande immunologiska och virologiska förloppet. Cellulära immunaktiveringsmarkörer samt cytokin nivåer ökar under sjukdomen, och sjunker under effektiv HIV behandling. Frånvaro av immunaktivering samt sjukdomsutveckling hos apor som är naturliga värdar av apviruset SIV och som inte blir sjuka förstärker ytterligare denna teori.

Huvudsyftet med denna avhandling var att karakterisera immunaktiveringen hos HIV- infekterade patienter och kartlägga betydelsen av proteinet ”high mobility group box protein 1” (HMGB1) i denna process. Därför har jag studerat: a) två unika patient kohorter. Den ena bestod av fyra patienter som genomgått levertransplantation och som behandlades med immundämpande läkemedel samt anti-HIV läkemedel. Den andra bestod av 22 patienter med HIV-infektion i tidig fas som fick anti-retroviral behandling och genomgick så kallad ’’analytical treatment interruption’’; b) interaktioner mellan HIV-1 och HMGB1 *in vitro* och *in vivo*.

Den antiretrovirala terapin som de HIV-infekterade levertransplanterade individerna erhöll var effektiv och ledde till odetekterbara virusnivåer samt få virusmutationer. Dessutom tydde förändringarna av virusnivåerna under den tidiga fasen av HIV-infektionen hos en av dessa patienter på att den initiala virus minskningen i plasma sannolikt var orsakad av det medfödda immunförsvaret eller reducerad immunoaktivering. I gruppen med 22 patienter med HIV-infektion i tidig fas var låg immunoaktivering korrelerat till lägre virusförökning samt bättre virus kontroll 2 år senare. Vi kunde också bekräfta att primär HIV-infektion karakteriseras av generaliserad immunoaktivering.

HMGB1 är ett protein som finns i cellkärnan i alla kroppens celler. Detta protein verkar både intra- och extracellulärt. Extracellulärt HMGB1, fungerar som en proinflammatorisk cytokin och kan stimulera ett stort antal celler. Proteinet har sannolikt en central roll i inflammatoriska reaktioner vid ett stort antal sjukdomstillstånd. Våra data visar att HMGB1 har stor betydelse vid HIV-infektion. Intracellulärt HMGB1 hämmade HIV´s förökning i vissa cellslag, bland annat monocyter. Vår tolkning är att en hög produktion av HMGB1 i denna celltyp leder till en låggradig virusförökning i cellerna och därmed utgör de en viktig reservoar för HIV-1. Vi kunde dessutom visa att HMGB1 tillsatt extracellulärt kunde modulera HIV´1s förökning i två cellsystemsmodeller för kronisk och akut HIV infektion. Sålunda ökade virusförökningen i celler (U1 celler) som är en modell för latent, sovande, HIV-1 infektion och hämmade HIV-1 i akut infekterade makrofager. Den senare effekten kunde förklaras av att vissa signalmolekyler (Β-kemokiner; RANTES, MIP-1a, MIP-1b) utsöndrades efter HMGB1 stimulering. Vi har också visat att HIV infekterade patienter har förhöjda plasma nivåer av HMGB1 med högsta koncentrationerna hos personer med kliniska komplikationer.

Samanfattningsvis, denna avhandling betonar vikten av immunoaktivering i sjukdomsutvecklingen vid HIV-infektion. HMGB1 är en viktig faktor i patogenesen. Proteinet påverkar HIV-förökningen samt leder till att immunsystemet inte fungerar adekvat. Fortsatta studier bör utvärdera om anti-HMGB1 läkemedel kan bidra till en normalisering av det hyperaktiva immunsystemet vid HIV infektion.

Acknowledgements

I wish to express my sincere thanks to all who have contributed to this work, especially to:

My supervisor Professor Anders Sönnerborg; Thank you for your generosity, the scientific guidance, encouragement and continuous support to my ideas throughout the study period. Especially for the confidence in me and the results during the ‘’western HMGB1’’ period.

Professor Anders Vahlne for giving me the opportunity to work at the division of clinical virology.

Dr. Andrzej Piasek for ‘’believing in me’’ from the beginning, his friendship and sharing his enthusiastic dedication to life and science. Other members of the AIDS Diagnosis and Therapy Center in Warsaw for giving me opportunity to start my work there and assistance in lab and fun activities.

My co-authors for fruitful collaborations.

Professor Jan Andersson and Calle Treutiger for collaboration in the extracellular HMGB1 project. Members of Ulf Andersson’s group especially Helena and Heidi for sharing the interest in the truly way that HMGB1 protein works.

Babbi the friend and collaborator. For trust in my experimental design and outstanding performance …as well as constructive criticism. We have at least few of the ‘’possible mechanisms’ not tested..

Samir, ‘’the guy who knows how it works’’ for unlimited answers to my questions, help with experiments optimization as well as for the scientific traveling and taking part in all intelligent and less intelligent activities.

All the other past and present members of ‘’Sönnerborgs group’’: Marcus (for the first welcome words), Annika (for introduction to top-class lab work) Soo, Annica-Eva (ladies), Tobias and Halime.

All former and present friends and colleagues at the F68; especially
Una and Juris for the early days.

CIMers : Maire, Mikolaj (intro into the apoptosis world), Annelie, Anna S, Lillian and Hernan

My friends in Warsaw (especially ‘’Jelonki team’’ + Tomek + AL) for all the fun during short and unexpected getting together.

My friends from Rzeszow (Madej, Jano, Bugs and Jaca + Krakow site) for always having time for me and help during the visits.

My Polish links in Sweden: Radek, Filip and Ela.

The extended Nowak and Schmidt families; Mama i Tato za moja edukacje i wychowanie.

Alex, for filling my life with endless love.

Kosmo and Anja for changing the dimensions of my journey……

This work was supported by grants from Karolinska Institute–Medical University of Warsaw Research Training Program (KIRT-MUW), Swedish Physicians Against AIDS Research Foundation, Swedish Research Council and SIDA /SAREC.

References

1. UNAIDS. AIDS 2006 Raport on the global epidemic. www.unaids.org 2006

2. Gottlieb MS, Schroff R, Schanker HM, et al. Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. N Engl J Med 1981;305:1425-31

3. Siegal FP, Lopez C, Hammer GS, et al. Severe acquired immunodeficiency in male homosexuals, manifested by chronic perianal ulcerative herpes simplex lesions. N Engl J Med 1981;305:1439-44

4. Masur H, Michelis MA, Greene JB, et al. An outbreak of community-acquired Pneumocystis carinii pneumonia: initial manifestation of cellular immune dysfunction. N Engl J Med 1981;305:1431-8

5. Barre-Sinoussi F, Chermann JC, Rey F, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 1983;220:868-71

6. Levy JA, Hoffman AD, Kramer SM, Landis JA, Shimabukuro JM and Oshiro LS. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. Science 1984;225:840-2

7. Popovic M, Sarngadharan MG, Read E and Gallo RC. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science 1984;224:497-500

8. Clavel F, Guetard D, Brun-Vezinet F, et al. Isolation of a new human retrovirus from West African patients with AIDS. Science 1986;233:343-6

9. Fields BN KD, Howley PM et al. Fields Virology Third edition. Philadelphia: Lippincott-Raven Publisher, 1996

10. Marlink R, Kanki P, Thior I, et al. Reduced rate of disease development after HIV-2 infection as compared to HIV-1. Science 1994;265:1587-90

11. Wyatt R, Sodroski J. The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. Science 1998;280:1884-8

12. Wyma DJ, Kotov A and Aiken C. Evidence for a stable interaction of gp41 with Pr55(Gag) in immature human immunodeficiency virus type 1 particles. J Virol 2000;74:9381-7

13. Hoglund S, Ofverstedt LG, Nilsson A, et al. Spatial visualization of the maturing HIV-1 core and its linkage to the envelope. AIDS Res Hum Retroviruses 1992;8:1-7

14. Frankel AD, Young JA. HIV-1: fifteen proteins and an RNA. Annu Rev Biochem 1998;67:1-25

15. Stevenson M. HIV-1 pathogenesis. Nat Med 2003;9:853-60

16. Ott DE. Potential roles of cellular proteins in HIV-1. Rev Med Virol 2002;12:359-74

17. Stevenson M. Developments in basic science research. Top HIV Med 2006;14:4-7

18. Klatzmann D, Barre-Sinoussi F, Nugeyre MT, et al. Selective tropism of lymphadenopathy associated virus (LAV) for helper-inducer T lymphocytes. Science 1984;225:59-63

19. Ho DD, Rota TR and Hirsch MS. Infection of monocyte/macrophages by human T lymphotropic virus type III. J Clin Invest 1986;77:1712-5

20. Patterson S, Knight SC. Susceptibility of human peripheral blood dendritic cells to infection by human immunodeficiency virus. J Gen Virol 1987;68 (Pt 4):1177-81

21. Berger EA, Murphy PM and Farber JM. Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. Annu Rev Immunol 1999;17:657-700

22. Doranz BJ, Berson JF, Rucker J and Doms RW. Chemokine receptors as fusion cofactors for human immunodeficiency virus type 1 (HIV-1). Immunol Res 1997;16:15-28

23. Doms RW, Moore JP. HIV-1 membrane fusion: targets of opportunity. J Cell Biol 2000;151:F9-14

24. Sherman MP, Greene WC. Slipping through the door: HIV entry into the nucleus. Microbes Infect 2002;4:67-73

25. Miller MD, Bushman FD. HIV integration. Ini1 for integration? Curr Biol 1995;5:368-70

26. Cullen BR, Greene WC. Regulatory pathways governing HIV-1 replication. Cell 1989;58:423-6

27. Lawn SD, Butera ST and Folks TM. Contribution of immune activation to the pathogenesis and transmission of human immunodeficiency virus type 1 infection. Clin Microbiol Rev 2001;14:753-77, table of contents

28. Resh MD. Intracellular trafficking of HIV-1 Gag: how Gag interacts with cell membranes and makes viral particles. AIDS Rev 2005;7:84-91

29. Nguyen DH, Hildreth JE. Evidence for budding of human immunodeficiency virus type 1 selectively from glycolipid-enriched membrane lipid rafts. J Virol 2000;74:3264-72

30. Kaplan AH, Manchester M and Swanstrom R. The activity of the protease of human immunodeficiency virus type 1 is initiated at the membrane of infected cells before the release of viral proteins and is required for release to occur with maximum efficiency. J Virol 1994;68:6782-6

31. Stevens M, De Clercq E and Balzarini J. The regulation of HIV-1 transcription: molecular targets for chemotherapeutic intervention. Med Res Rev 2006;26:595-625

32. Krebs F HT, Quiterio S, Gartner S, Wigdahl B. Lentiviral LTR-directed expression, sequence variation and disease pathogenesis. Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM, LA-UR 02-2877., 2001 (2001. iHSC, ed.

33. Klaver B, Berkhout B. Comparison of 5' and 3' long terminal repeat promoter function in human immunodeficiency virus. J Virol 1994;68:3830-40

34. Verdin E, Paras P, Jr. and Van Lint C. Chromatin disruption in the promoter of human immunodeficiency virus type 1 during transcriptional activation. Embo J 1993;12:3249-59

35. Pereira LA, Bentley K, Peeters A, Churchill MJ and Deacon NJ. A compilation of cellular transcription factor interactions with the HIV-1 LTR promoter. Nucleic Acids Res 2000;28:663-8

36. Jones KA, Luciw PA and Duchange N. Structural arrangements of transcription control domains within the 5'-untranslated leader regions of the HIV-1 and HIV-2 promoters. Genes Dev 1988;2:1101-14

37. Harrich D, Garcia J, Wu F, Mitsuyasu R, Gonazalez J and Gaynor R. Role of SP1-binding domains in in vivo transcriptional regulation of the human immunodeficiency virus type 1 long terminal repeat. J Virol 1989;63:2585-91

38. Harrich D, Hooker CW and Parry E. The human immunodeficiency virus type 1 TAR RNA upper stem-loop plays distinct roles in reverse transcription and RNA packaging. J Virol 2000;74:5639-46

39. Jones KA, Kadonaga JT, Luciw PA and Tjian R. Activation of the AIDS retrovirus promoter by the cellular transcription factor, Sp1. Science 1986;232:755-9

40. Garcia JA, Harrich D, Soultanakis E, Wu F, Mitsuyasu R and Gaynor RB. Human immunodeficiency virus type 1 LTR TATA and TAR region sequences required for transcriptional regulation. Embo J 1989;8:765-78

41. Grilli M, Chiu JJ and Lenardo MJ. NF-kappa B and Rel: participants in a multiform transcriptional regulatory system. Int Rev Cytol 1993;143:1-62

42. Rabson AB, Lin HC. NF-kappa B and HIV: linking viral and immune activation. Adv Pharmacol 2000;48:161-207

43. Lenardo MJ, Baltimore D. NF-kappa B: a pleiotropic mediator of inducible and tissue-specific gene control. Cell 1989;58:227-9

44. Chen BK, Feinberg MB and Baltimore D. The kappaB sites in the human immunodeficiency virus type 1 long terminal repeat enhance virus replication yet are not absolutely required for viral growth. J Virol 1997;71:5495-504

45. Parrott C, Seidner T, Duh E, et al. Variable role of the long terminal repeat Sp1-binding sites in human immunodeficiency virus replication in T lymphocytes. J Virol 1991;65:1414-9

46. Lu Y, Stenzel M, Sodroski JG and Haseltine WA. Effects of long terminal repeat mutations on human immunodeficiency virus type 1 replication. J Virol 1989;63:4115-9

47. Lu YC, Touzjian N, Stenzel M, Dorfman T, Sodroski JG and Haseltine WA. Identification of cis-acting repressive sequences within the negative regulatory element of human immunodeficiency virus type 1. J Virol 1990;64:5226-9

48. Schwartz S, Felber BK, Benko DM, Fenyo EM and Pavlakis GN. Cloning and functional analysis of multiply spliced mRNA species of human immunodeficiency virus type 1. J Virol 1990;64:2519-29

49. Rohr O, Marban C, Aunis D and Schaeffer E. Regulation of HIV-1 gene transcription: from lymphocytes to microglial cells. J Leukoc Biol 2003;74:736-49

50. Liang C, Wainberg MA. The role of Tat in HIV-1 replication: an activator and/or a suppressor? AIDS Rev 2002;4:41-9

51. Conant K, Ma M, Nath A and Major EO. Extracellular human immunodeficiency virus type 1 Tat protein is associated with an increase in both NF-kappa B binding and protein kinase C activity in primary human astrocytes. J Virol 1996;70:1384-9

52. Demarchi F, d'Adda di Fagagna F, Falaschi A and Giacca M. Activation of transcription factor NF-kappaB by the Tat protein of human immunodeficiency virus type 1. J Virol 1996;70:4427-37

53. Nowak MA. Variability of HIV infections. J Theor Biol 1992;155:1-20

54. Goodenow M, Huet T, Saurin W, Kwok S, Sninsky J and Wain-Hobson S. HIV-1 isolates are rapidly evolving quasispecies: evidence for viral mixtures and preferred nucleotide substitutions. J Acquir Immune Defic Syndr 1989;2:344-52

55. Preston BD, Poiesz BJ and Loeb LA. Fidelity of HIV-1 reverse transcriptase. Science 1988;242:1168-71

56. Roberts JD, Bebenek K and Kunkel TA. The accuracy of reverse transcriptase from HIV-1. Science 1988;242:1171-3

57. Ricchetti M, Buc H. Reverse transcriptases and genomic variability: the accuracy of DNA replication is enzyme specific and sequence dependent. Embo J 1990;9:1583-93

58. Perelson AS, Neumann AU, Markowitz M, Leonard JM and Ho DD. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. Science 1996;271:1582-6

59. Korber BT, Allen EE, Farmer AD and Myers GL. Heterogeneity of HIV-1 and HIV-2. Aids 1995;9 Suppl A:S5-18

60. Brown AL, Monaghan P. Evolution of the structural proteins of human immunodeficiency virus: selective constraints on nucleotide substitution. AIDS Res Hum Retroviruses 1988;4:399-407

61. Leitner T, Kumar S and Albert J. Tempo and mode of nucleotide substitutions in gag and env gene fragments in human immunodeficiency virus type 1 populations with a known transmission history. J Virol 1997;71:4761-70

62. Leitner T, Albert J. The molecular clock of HIV-1 unveiled through analysis of a known transmission history. Proc Natl Acad Sci U S A 1999;96:10752-7

63. Starcich BR, Hahn BH, Shaw GM, et al. Identification and characterization of conserved and variable regions in the envelope gene of HTLV-III/LAV, the retrovirus of AIDS. Cell 1986;45:637-48

64. Palker TJ, Matthews TJ, Langlois A, et al. Polyvalent human immunodeficiency virus synthetic immunogen comprised of envelope gp120 T helper cell sites and B cell neutralization epitopes. J Immunol 1989;142:3612-9

65. Nara PL, Garrity RR and Goudsmit J. Neutralization of HIV-1: a paradox of humoral proportions. Faseb J 1991;5:2437-55

66. Takahashi H, Nakagawa Y, Pendleton CD, et al. Induction of broadly cross-reactive cytotoxic T cells recognizing an HIV-1 envelope determinant. Science 1992;255:333-6

67. Hwang SS, Boyle TJ, Lyerly HK and Cullen BR. Identification of the envelope V3 loop as the primary determinant of cell tropism in HIV-1. Science 1991;253:71-4

68. de Jong JJ, Goudsmit J, Keulen W, et al. Human immunodeficiency virus type 1 clones chimeric for the envelope V3 domain differ in syncytium formation and replication capacity. J Virol 1992;66:757-65

69. Tatt ID, Barlow KL, Clewley JP, Gill ON and Parry JV. Surveillance of HIV-1 subtypes among heterosexuals in England and Wales, 1997-2000. J Acquir Immune Defic Syndr 2004;36:1092-9

70. Zhu T, Mo H, Wang N, et al. Genotypic and phenotypic characterization of HIV-1 patients with primary infection. Science 1993;261:1179-81

71. Karlsson AC, Lindback S, Gaines H and Sonnerborg A. Characterization of the viral population during primary HIV-1 infection. Aids 1998;12:839-47

72. Nowak P, Karlsson AC, Naver L, Bohlin AB, Piasek A and Sonnerborg A. The selection and evolution of viral quasispecies in HIV-1 infected children. HIV Med 2002;3:1-11

73. Miedema F, Tersmette M and van Lier RA. AIDS pathogenesis: a dynamic interaction between HIV and the immune system. Immunol Today 1990;11:293-7

74. Harrer T, Harrer E, Kalams SA, et al. Cytotoxic T lymphocytes in asymptomatic long-term nonprogressing HIV-1 infection. Breadth and specificity of the response and relation to in vivo viral quasispecies in a person with prolonged infection and low viral load. J Immunol 1996;156:2616-23

75. Castiglione F, Poccia F, D'Offizi G and Bernaschi M. Mutation, fitness, viral diversity, and predictive markers of disease progression in a computational model of HIV type 1 infection. AIDS Res Hum Retroviruses 2004;20:1314-23

76. Shankarappa R, Margolick JB, Gange SJ, et al. Consistent viral evolutionary changes associated with the progression of human immunodeficiency virus type 1 infection. J Virol 1999;73:10489-502

77. Nowak MA, Anderson RM, McLean AR, Wolfs TF, Goudsmit J and May RM. Antigenic diversity thresholds and the development of AIDS. Science 1991;254:963-9

78. Cooper DA, Gold J, Maclean P, et al. Acute AIDS retrovirus infection. Definition of a clinical illness associated with seroconversion. Lancet 1985;1:537-40

79. Kinloch-de Loes S, de Saussure P, Saurat JH, Stalder H, Hirschel B and Perrin LH. Symptomatic primary infection due to human immunodeficiency virus type 1: review of 31 cases. Clin Infect Dis 1993;17:59-65

80. Lindback S, Thorstensson R, Karlsson AC, et al. Diagnosis of primary HIV-1 infection and duration of follow-up after HIV exposure. Karolinska Institute Primary HIV Infection Study Group. Aids 2000;14:2333-9

81. Brenchley JM, Price DA and Douek DC. HIV disease: fallout from a mucosal catastrophe? Nat Immunol 2006;7:235-9

82. Lindback S, Karlsson AC, Mittler J, et al. Viral dynamics in primary HIV-1 infection. Karolinska Institutet Primary HIV Infection Study Group. Aids 2000;14:2283-91

83. Pantaleo G, Demarest JF, Schacker T, et al. The qualitative nature of the primary immune response to HIV infection is a prognosticator of disease progression independent of the initial level of plasma viremia. Proc Natl Acad Sci U S A 1997;94:254-8

84. Lefrere JJ, Roudot-Thoraval F, Mariotti M, et al. The risk of disease progression is determined during the first year of human immunodeficiency virus type 1 infection. J Infect Dis 1998;177:1541-8

85. Hel Z, McGhee JR and Mestecky J. HIV infection: first battle decides the war. Trends Immunol 2006;27:274-81

86. Mellors JW, Munoz A, Giorgi JV, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. Ann Intern Med 1997;126:946-54

87. Jaffar S, Grant AD, Whitworth J, Smith PG and Whittle H. The natural history of HIV-1 and HIV-2 infections in adults in Africa: a literature review. Bull World Health Organ 2004;82:462-9

88. Lyles RH, Munoz A, Yamashita TE, et al. Natural history of human immunodeficiency virus type 1 viremia after seroconversion and proximal to AIDS in a large cohort of homosexual men. Multicenter AIDS Cohort Study. J Infect Dis 2000;181:872-80

89. Jones JL, Hanson DL, Dworkin MS, et al. Surveillance for AIDS-defining opportunistic illnesses, 1992-1997. MMWR CDC Surveill Summ 1999;48:1-22

90. Richman DD. HIV chemotherapy. Nature 2001;410:995-1001

91. Sharp M. XVI International AIDS Conference. New drug classes from Toronto. Plus some older ones. Posit Aware 2006;17:16-7

92. Citterio P, Rusconi S. Novel inhibitors of the early steps of the HIV-1 life cycle. Expert Opin Investig Drugs 2007;16:11-23

93. Autran B, Carcelain G, Li TS, et al. Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. Science 1997;277:112-6

94. Aleman S, Pehrson P and Sonnerborg A. Kinetics of beta-chemokine levels during anti-HIV therapy. Antivir Ther 1999;4:109-15

95. Palella FJ, Jr., Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med 1998;338:853-60

96. Finzi D, Hermankova M, Pierson T, et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science 1997;278:1295-300

97. Pierson T, McArthur J and Siliciano RF. Reservoirs for HIV-1: mechanisms for viral persistence in the presence of antiviral immune responses and antiretroviral therapy. Annu Rev Immunol 2000;18:665-708

98. Loutfy MR, Walmsley SL. Salvage antiretroviral therapy in HIV infection. Expert Opin Pharmacother 2002;3:81-90

99. Bisson G, Gross R, Miller V, et al. Monitoring of long-term toxicities of HIV treatments: an international perspective. Aids 2003;17:2407-17

100. Kulasekaram R, Peters BS and Wierzbicki AS. Dyslipidaemia and cardiovascular risk in HIV infection. Curr Med Res Opin 2005;21:1717-25

101. Lori F, Lisziewicz J. Structured treatment interruptions for the management of HIV infection. Jama 2001;286:2981-7

102. Montaner LJ. Structured treatment interruptions to control HIV-1 and limit drug exposure. Trends Immunol 2001;22:92-6

103. El-Sadr WM, Lundgren JD, Neaton JD, et al. CD4+ count-guided interruption of antiretroviral treatment. N Engl J Med 2006;355:2283-96

104. Currier JS, Baden LR. Getting smarter--the toxicity of undertreated HIV infection. N Engl J Med 2006;355:2359-61

105. Grossman Z, Meier-Schellersheim M, Paul WE and Picker LJ. Pathogenesis of HIV infection: what the virus spares is as important as what it destroys. Nat Med 2006;12:289-95

106. Brenchley JM, Hill BJ, Ambrozak DR, et al. T-cell subsets that harbor human immunodeficiency virus (HIV) in vivo: implications for HIV pathogenesis. J Virol 2004;78:1160-8

107. Douek DC, Picker LJ and Koup RA. T cell dynamics in HIV-1 infection. Annu Rev Immunol 2003;21:265-304

108. Appay V, Boutboul F and Autran B. The HIV infection and immune activation: "to fight and burn". Curr Infect Dis Rep 2005;7:473-9

109. Maggi P, Maserati R and Antonelli G. Atherosclerosis in HIV patients: a new face for an old disease? AIDS Rev 2006;8:204-9

110. Deeks SG, Kitchen CM, Liu L, et al. Immune activation set point during early HIV infection predicts subsequent CD4+ T-cell changes independent of viral load. Blood 2004;104:942-7

111. Nilsson J, Kinloch-de-Loes S, Granath A, Sonnerborg A, Goh LE and Andersson J. Early immune activation in gut-associated and peripheral lymphoid tissue during acute HIV infection. Aids 2007;21:565-574

112. Moanna A, Dunham R, Paiardini M and Silvestri G. CD4+ T-cell depletion in HIV infection: killed by friendly fire? Curr HIV/AIDS Rep 2005;2:16-23

113. Tesselaar K, Arens R, van Schijndel GM, et al. Lethal T cell immunodeficiency induced by chronic costimulation via CD27-CD70 interactions. Nat Immunol 2003;4:49-54

114. Silvestri G, Sodora DL, Koup RA, et al. Nonpathogenic SIV infection of sooty mangabeys is characterized by limited bystander immunopathology despite chronic high-level viremia. Immunity 2003;18:441-52

115. Clerici M, Declich S and Rizzardini G. African enigma: key player in human immunodeficiency virus pathogenesis in developing countries? Clin Diagn Lab Immunol 2001;8:864-6

116. Pisell TL, Hoffman IF, Jere CS, et al. Immune activation and induction of HIV-1 replication within CD14 macrophages during acute Plasmodium falciparum malaria coinfection. Aids 2002;16:1503-9

117. Rizzardini G, Trabattoni D, Saresella M, et al. Immune activation in HIV-infected African individuals. Italian-Ugandan AIDS cooperation program. Aids 1998;12:2387-96

118. Dyer JR, Hoffman IF, Eron JJ, Jr., Fiscus SA and Cohen MS. Immune activation and plasma viral load in HIV-infected African individuals. Aids 1999;13:1283-5

119. Dyer JR, Kazembe P, Vernazza PL, et al. High levels of human immunodeficiency virus type 1 in blood and semen of seropositive men in sub-Saharan Africa. J Infect Dis 1998;177:1742-6

120. Lawn SD. AIDS in Africa: the impact of coinfections on the pathogenesis of HIV-1 infection. J Infect 2004;48:1-12

121. Israel-Biet D, Cadranel J and Even P. Human immunodeficiency virus production by alveolar lymphocytes is increased during Pneumocystis carinii pneumonia. Am Rev Respir Dis 1993;148:1308-12

122. Wiktor SZ, Sassan-Morokro M, Grant AD, et al. Efficacy of trimethoprim-sulphamethoxazole prophylaxis to decrease morbidity and mortality in HIV-1-infected patients with tuberculosis in Abidjan, Cote d'Ivoire: a randomised controlled trial. Lancet 1999;353:1469-75

123. Stanley SK, Ostrowski MA, Justement JS, et al. Effect of immunization with a common recall antigen on viral expression in patients infected with human immunodeficiency virus type 1. N Engl J Med 1996;334:1222-30

124. Sousa AE, Carneiro J, Meier-Schellersheim M, Grossman Z and Victorino RM. CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. J Immunol 2002;169:3400-6

125. Hazenberg MD, Otto SA, van Benthem BH, et al. Persistent immune activation in HIV-1 infection is associated with progression to AIDS. Aids 2003;17:1881-8

126. Savarino A, Bottarel F, Malavasi F and Dianzani U. Role of CD38 in HIV-1 infection: an epiphenomenon of T-cell activation or an active player in virus/host interactions? Aids 2000;14:1079-89

127. Giorgi JV, Lyles RH, Matud JL, et al. Predictive value of immunologic and virologic markers after long or short duration of HIV-1 infection. J Acquir Immune Defic Syndr 2002;29:346-55

128. Kvale D, Aukrust P, Osnes K, Muller F and Froland SS. CD4+ and CD8+ lymphocytes and HIV RNA in HIV infection: high baseline counts and in particular rapid decrease of CD8+ lymphocytes predict AIDS. Aids 1999;13:195-201

129. Mohri H, Perelson AS, Tung K, et al. Increased turnover of T lymphocytes in HIV-1 infection and its reduction by antiretroviral therapy. J Exp Med 2001;194:1277-87

130. Ameisen JC, Capron A. Cell dysfunction and depletion in AIDS: the programmed cell death hypothesis. Immunol Today 1991;12:102-5

131. Gougeon ML, Lecoeur H, Dulioust A, et al. Programmed cell death in peripheral lymphocytes from HIV-infected persons: increased susceptibility to apoptosis of CD4 and CD8 T cells correlates with lymphocyte activation and with disease progression. J Immunol 1996;156:3509-20

132. Finkel TH, Tudor-Williams G, Banda NK, et al. Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. Nat Med 1995;1:129-34

133. Poli G, Fauci AS. The effect of cytokines and pharmacologic agents on chronic HIV infection. AIDS Res Hum Retroviruses 1992;8:191-7

134. Clerici M, Shearer GM. A TH1-->TH2 switch is a critical step in the etiology of HIV infection. Immunol Today 1993;14:107-11

135. Kinter A, Arthos J, Cicala C and Fauci AS. Chemokines, cytokines and HIV: a complex network of interactions that influence HIV pathogenesis. Immunol Rev 2000;177:88-98

136. Kedzierska K, Crowe SM. Cytokines and HIV-1: interactions and clinical implications. Antivir Chem Chemother 2001;12:133-50

137. Zangerle R, Gallati H, Sarcletti M, et al. Increased serum concentrations of soluble tumor necrosis factor receptors in HIV-infected individuals are associated with immune activation. J Acquir Immune Defic Syndr 1994;7:79-85

138. Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC and Lusso P. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. Science 1995;270:1811-5

139. Alfano M, Poli G. Role of cytokines and chemokines in the regulation of innate immunity and HIV infection. Mol Immunol 2005;42:161-82

140. Kumar D, Parato K, Kumar A, Sun E, Cameron DW and Angel JB. Sustained suppression of plasma HIV RNA is associated with an increase in the production of mitogen-induced MIP-1alpha and MIP-1beta. AIDS Res Hum Retroviruses 1999;15:1073-7

141. Polo S, Veglia F, Malnati MS, et al. Longitudinal analysis of serum chemokine levels in the course of HIV-1 infection. Aids 1999;13:447-54

142. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med 2006;12:1365-71

143. Haynes BF. Gut microbes out of control in HIV infection. Nat Med 2006;12:1351-2

144. Hazenberg MD, Stuart JW, Otto SA, et al. T-cell division in human immunodeficiency virus (HIV)-1 infection is mainly due to immune activation: a longitudinal analysis in patients before and during highly active antiretroviral therapy (HAART). Blood 2000;95:249-55

145. Davey RT, Jr., Bhat N, Yoder C, et al. HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. Proc Natl Acad Sci U S A 1999;96:15109-14

146. Valdez H, Connick E, Smith KY, et al. Limited immune restoration after 3 years' suppression of HIV-1 replication in patients with moderately advanced disease. Aids 2002;16:1859-66

147. Lempicki RA, Kovacs JA, Baseler MW, et al. Impact of HIV-1 infection and highly active antiretroviral therapy on the kinetics of CD4+ and CD8+ T cell turnover in HIV-infected patients. Proc Natl Acad Sci U S A 2000;97:13778-83

148. Chapuis AG, Paolo Rizzardi G, D'Agostino C, et al. Effects of mycophenolic acid on human immunodeficiency virus infection in vitro and in vivo. Nat Med 2000;6:762-8

149. Romanelli F, Hoven AD. Use of virostatics as a means of targeting human immunodeficiency virus infection. Curr Pharm Des 2006;12:1121-7

150. Jacobson JM. Immune-based therapies: an adjunct to antiretroviral treatment. Curr HIV/AIDS Rep 2005;2:90-7

151. Ho S, Clipstone N, Timmermann L, et al. The mechanism of action of cyclosporin A and FK506. Clin Immunol Immunopathol 1996;80:S40-5

152. Briggs CJ, Ott DE, Coren LV, Oroszlan S and Tozser J. Comparison of the effect of FK506 and cyclosporin A on virus production in H9 cells chronically and newly infected by HIV-1. Arch Virol 1999;144:2151-60

153. Rizzardi GP, Harari A, Capiluppi B, et al. Treatment of primary HIV-1 infection with cyclosporin A coupled with highly active antiretroviral therapy. J Clin Invest 2002;109:681-8

154. Khonkarly M TG, Ciuffreda D, et al. Long-term benefit of cyclosporin A coupled with highly active retroviral therapy in primary HIV-1 infection (abstract 567). In: In : Program and abstracts of the 12th Conference on Retroviruses and Opprotunistic Infections Boston 2005

155. Lederman MM, Smeaton L, Smith KY, et al. Cyclosporin A provides no sustained immunologic benefit to persons with chronic HIV-1 infection starting suppressive antiretroviral therapy: results of a randomized, controlled trial of the AIDS Clinical Trials Group A5138. J Infect Dis 2006;194:1677-85

156. Hunt PW, Deeks SG. Immune-based therapy for HIV infection: are acute and chronic HIV infection different diseases? J Infect Dis 2006;194:1632-4

157. Goodwin GH, Sanders C and Johns EW. A new group of chromatin-associated proteins with a high content of acidic and basic amino acids. Eur J Biochem 1973;38:14-9

158. Bustin M. Revised nomenclature for high mobility group (HMG) chromosomal proteins. Trends Biochem Sci 2001;26:152-3

159. Bianchi ME, Agresti A. HMG proteins: dynamic players in gene regulation and differentiation. Curr Opin Genet Dev 2005;15:496-506

160. Mosevitsky MI, Novitskaya VA, Iogannsen MG and Zabezhinsky MA. Tissue specificity of nucleo-cytoplasmic distribution of HMG1 and HMG2 proteins and their probable functions. Eur J Biochem 1989;185:303-10

161. Agresti A, Bianchi ME. HMGB proteins and gene expression. Curr Opin Genet Dev 2003;13:170-8

162. West KL, Castellini MA, Duncan MK and Bustin M. Chromosomal proteins HMGN3a and HMGN3b regulate the expression of glycine transporter 1. Mol Cell Biol 2004;24:3747-56

163. Ferrari S, Finelli P, Rocchi M and Bianchi ME. The active gene that encodes human high mobility group 1 protein (HMG1) contains introns and maps to chromosome 13. Genomics 1996;35:367-71

164. Ulloa L, Messmer D. High-mobility group box 1 (HMGB1) protein: friend and foe. Cytokine Growth Factor Rev 2006;17:189-201

165. Agresti A, Lupo R, Bianchi ME and Muller S. HMGB1 interacts differentially with members of the Rel family of transcription factors. Biochem Biophys Res Commun 2003;302:421-6

166. Lotze MT, Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. Nat Rev Immunol 2005;5:331-42

167. Zeh HJ, 3rd, Lotze MT. Addicted to death: invasive cancer and the immune response to unscheduled cell death. J Immunother 2005;28:1-9

168. Calogero S, Grassi F, Aguzzi A, et al. The lack of chromosomal protein Hmg1 does not disrupt cell growth but causes lethal hypoglycaemia in newborn mice. Nat Genet 1999;22:276-80

169. Wang H, Bloom O, Zhang M, et al. HMG-1 as a late mediator of endotoxin lethality in mice. Science 1999;285:248-51

170. Hori O, Brett J, Slattery T, et al. The receptor for advanced glycation end products (RAGE) is a cellular binding site for amphoterin. Mediation of neurite outgrowth and co-expression of rage and amphoterin in the developing nervous system. J Biol Chem 1995;270:25752-61

171. Passalacqua M, Zicca A, Sparatore B, Patrone M, Melloni E and Pontremoli S. Secretion and binding of HMG1 protein to the external surface of the membrane are required for murine erythroleukemia cell differentiation. FEBS Lett 1997;400:275-9

172. Gardella S, Andrei C, Ferrera D, et al. The nuclear protein HMGB1 is secreted by monocytes via a non-classical, vesicle-mediated secretory pathway. EMBO Rep 2002;3:995-1001

173. Bonaldi T, Talamo F, Scaffidi P, et al. Monocytic cells hyperacetylate chromatin protein HMGB1 to redirect it towards secretion. Embo J 2003;22:5551-60

174. Scaffidi P, Misteli T and Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature 2002;418:191-5

175. Rovere-Querini P, Capobianco A, Scaffidi P, et al. HMGB1 is an endogenous immune adjuvant released by necrotic cells. EMBO Rep 2004;5:825-30

176. Harris HE, Raucci A. Alarmin(g) news about danger: workshop on innate danger signals and HMGB1. EMBO Rep 2006;7:774-8

177. Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. J Leukoc Biol 2007;81:1-5

178. Andersson U, Wang H, Palmblad K, et al. High mobility group 1 protein (HMG-1) stimulates proinflammatory cytokine synthesis in human monocytes. J Exp Med 2000;192:565-70

179. Parrish W, Ulloa L. High-mobility group box-1 isoforms as potential therapeutic targets in sepsis. Methods Mol Biol 2007;361:145-62

180. Andersson U, Erlandsson-Harris H, Yang H and Tracey KJ. HMGB1 as a DNA-binding cytokine. J Leukoc Biol 2002;72:1084-91

181. Mitola S, Belleri M, Urbinati C, et al. Cutting edge: extracellular high mobility group box-1 protein is a proangiogenic cytokine. J Immunol 2006;176:12-5

182. Huttunen HJ, Rauvala H. Amphoterin as an extracellular regulator of cell motility: from discovery to disease. J Intern Med 2004;255:351-66

183. Dumitriu IE, Bianchi ME, Bacci M, Manfredi AA and Rovere-Querini P. The secretion of HMGB1 is required for the migration of maturing dendritic cells. J Leukoc Biol 2007;81:84-91

184. Yang D, Chen Q, Yang H, Tracey KJ, Bustin M and Oppenheim JJ. High mobility group box-1 protein induces the migration and activation of human dendritic cells and acts as an alarmin. J Leukoc Biol 2007;81:59-66

185. Limana F, Germani A, Zacheo A, et al. Exogenous high-mobility group box 1 protein induces myocardial regeneration after infarction via enhanced cardiac C-kit+ cell proliferation and differentiation. Circ Res 2005;97:e73-83

186. Huttunen HJ, Fages C and Rauvala H. Receptor for advanced glycation end products (RAGE)-mediated neurite outgrowth and activation of NF-kappaB require the cytoplasmic domain of the receptor but different downstream signaling pathways. J Biol Chem 1999;274:19919-24

187. Ramasamy R, Vannucci SJ, Yan SS, Herold K, Yan SF and Schmidt AM. Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation. Glycobiology 2005;15:16R-28R

188. Rong LL, Gooch C, Szabolcs M, et al. RAGE: a journey from the complications of diabetes to disorders of the nervous system - striking a fine balance between injury and repair. Restor Neurol Neurosci 2005;23:355-65

189. Park JS, Gamboni-Robertson F, He Q, et al. High mobility group box 1 protein interacts with multiple Toll-like receptors. Am J Physiol Cell Physiol 2006;290:C917-24

190. Wang H, Yang H and Tracey KJ. Extracellular role of HMGB1 in inflammation and sepsis. J Intern Med 2004;255:320-31

191. Yang H, Wang H, Czura CJ and Tracey KJ. The cytokine activity of HMGB1. J Leukoc Biol 2005;78:1-8

192. Mantell LL, Parrish WR and Ulloa L. Hmgb-1 as a therapeutic target for infectious and inflammatory disorders. Shock 2006;25:4-11

193. Andersson U, Erlandsson-Harris H. HMGB1 is a potent trigger of arthritis. J Intern Med 2004;255:344-50

194. Hatada T, Wada H, Nobori T, et al. Plasma concentrations and importance of High Mobility Group Box protein in the prognosis of organ failure in patients with disseminated intravascular coagulation. Thromb Haemost 2005;94:975-9

195. Sunden-Cullberg J, Norrby-Teglund A, Rouhiainen A, et al. Persistent elevation of high mobility group box-1 protein (HMGB1) in patients with severe sepsis and septic shock. Crit Care Med 2005;33:564-73

196. Yasuda T, Ueda T, Takeyama Y, et al. Significant increase of serum high-mobility group box chromosomal protein 1 levels in patients with severe acute pancreatitis. Pancreas 2006;33:359-63

197. Sunden-Cullberg J, Norrby-Teglund A and Treutiger CJ. The role of high mobility group box-1 protein in severe sepsis. Curr Opin Infect Dis 2006;19:231-6

198. Urbonaviciute V, Furnrohr BG, Weber C, et al. Factors masking HMGB1 in human serum and plasma. J Leukoc Biol 2007;81:67-74

199. Rouhiainen A, Tumova S, Valmu L, Kalkkinen N and Rauvala H. Pivotal advance: analysis of proinflammatory activity of highly purified eukaryotic recombinant HMGB1 (amphoterin). J Leukoc Biol 2007;81:49-58

200. Muller S, Scaffidi P, Degryse B, et al. New EMBO members' review: the double life of HMGB1 chromatin protein: architectural factor and extracellular signal. Embo J 2001;20:4337-40

201. Tremethick DJ, Molloy PL. Stimulation of transcription from different RNA polymerase II promoters by high mobility group proteins 1 and 2. FEBS Lett 1989;242:346-50

202. Song MJ, Hwang S, Wong W, et al. The DNA architectural protein HMGB1 facilitates RTA-mediated viral gene expression in gamma-2 herpesviruses. J Virol 2004;78:12940-50

203. Zhang G, Kobayashi T, Kamitani W, et al. Borna disease virus phosphoprotein represses p53-mediated transcriptional activity by interference with HMGB1. J Virol 2003;77:12243-51

204. Finberg RW, Wang JP and Kurt-Jones EA. Toll like receptors and viruses. Rev Med Virol 2007;17:35-43

205. Wang H, Ward MF, Fan XG, Sama AE and Li W. Potential role of high mobility group box 1 in viral infectious diseases. Viral Immunol 2006;19:3-9

206. Chu JJ, Ng ML. The mechanism of cell death during West Nile virus infection is dependent on initial infectious dose. J Gen Virol 2003;84:3305-14

207. Joseph T, Cepica A, Brown L, Ikede BO and Kibenge FS. Mechanism of cell death during infectious salmon anemia virus infection is cell type-specific. J Gen Virol 2004;85:3027-36

208. Plymale DR, Tang DS, Comardelle AM, Fermin CD, Lewis DE and Garry RF. Both necrosis and apoptosis contribute to HIV-1-induced killing of CD4 cells. Aids 1999;13:1827-39

209. Van Reeth K. Cytokines in the pathogenesis of influenza. Vet Microbiol 2000;74:109-16

210. Ward SE, Loutfy MR, Blatt LM, et al. Dynamic changes in clinical features and cytokine/chemokine responses in SARS patients treated with interferon alfacon-1 plus corticosteroids. Antivir Ther 2005;10:263-75

211. Sass G, Heinlein S, Agli A, Bang R, Schumann J and Tiegs G. Cytokine expression in three mouse models of experimental hepatitis. Cytokine 2002;19:115-20

212. Sitia G, Iannacone M, Muller S, Bianchi ME and Guidotti LG. Treatment with HMGB1 inhibitors diminishes CTL-induced liver disease in HBV transgenic mice. J Leukoc Biol 2007;81:100-7

213. Rosenthal E, Pialoux G, Bernard N, et al. Liver-related mortality in human-immunodeficiency-virus-infected patients between 1995 and 2003 in the French GERMIVIC Joint Study Group Network (MORTAVIC 2003 Study)*. J Viral Hepat 2007;14:183-8

214. Gow PJ, Pillay D and Mutimer D. Solid organ transplantation in patients with HIV infection. Transplantation 2001;72:177-81

215. Jin X, Bauer DE, Tuttleton SE, et al. Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. J Exp Med 1999;189:991-8

216. Izzedine H, Launay-Vacher V, Baumelou A and Deray G. Antiretroviral and immunosuppressive drug-drug interactions: an update. Kidney Int 2004;66:532-41

217. Neff GW, Sherman KE, Eghtesad B and Fung J. Review article: current status of liver transplantation in HIV-infected patients. Aliment Pharmacol Ther 2004;20:993-1000

218. Schmitz JE, Kuroda MJ, Santra S, et al. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science 1999;283:857-60

219. Karlsson AC, Birk M, Lindback S, Gaines H, Mittler JE and Sonnerborg A. Initiation of therapy during primary HIV type 1 infection results in a continuous decay of proviral DNA and a highly restricted viral evolution. AIDS Res Hum Retroviruses 2001;17:409-16

220. Lehming N, Thanos D, Brickman JM, Ma J, Maniatis T and Ptashne M. An HMG-like protein that can switch a transcriptional activator to a repressor. Nature 1994;371:175-9

221. Canaple L, Decoville M, Leng M and Locker D. The Drosophila DSP1 gene encoding an HMG 1-like protein: genomic organization, evolutionary conservation and expression. Gene 1997;184:285-90

222. Lenardo MJ, Angleman SB, Bounkeua V, et al. Cytopathic killing of peripheral blood CD4(+) T lymphocytes by human immunodeficiency virus type 1 appears necrotic rather than apoptotic and does not require env. J Virol 2002;76:5082-93

223. Mondal D, Alam J and Prakash O. NF-kappa B site-mediated negative regulation of the HIV-1 promoter by CCAAT/enhancer binding proteins in brain-derived cells. J Mol Neurosci 1994;5:241-58

224. Hoover T, Mikovits J, Court D, Liu YL, Kung HF and Raziuddin. A nuclear matrix-specific factor that binds a specific segment of the negative regulatory element (NRE) of HIV-1 LTR and inhibits NF-kappa(B) activity. Nucleic Acids Res 1996;24:1895-900

225. Naghavi MH, Schwartz S, Sonnerborg A and Vahlne A. Long terminal repeat promoter/enhancer activity of different subtypes of HIV type 1. AIDS Res Hum Retroviruses 1999;15:1293-303

226. Cassol E, Alfano M, Biswas P and Poli G. Monocyte-derived macrophages and myeloid cell lines as targets of HIV-1 replication and persistence. J Leukoc Biol 2006;80:1018-30

227. Thierry S, Gozlan J, Jaulmes A, et al. High-mobility group box 1 protein induces HIV-1 expression from persistently infected cells. Aids 2007;21:283-92

228. Kedzierska K, Crowe SM, Turville S and Cunningham AL. The influence of cytokines, chemokines and their receptors on HIV-1 replication in monocytes and macrophages. Rev Med Virol 2003;13:39-56

229. Verani A, Scarlatti G, Comar M, et al. C-C chemokines released by lipopolysaccharide (LPS)-stimulated human macrophages suppress HIV-1 infection in both macrophages and T cells. J Exp Med 1997;185:805-16

230. Lane BR, Markovitz DM, Woodford NL, Rochford R, Strieter RM and Coffey MJ. TNF-alpha inhibits HIV-1 replication in peripheral blood monocytes and alveolar macrophages by inducing the production of RANTES and decreasing C-C chemokine receptor 5 (CCR5) expression. J Immunol 1999;163:3653-61

231. Creery D, Weiss W, Lim WT, Aziz Z, Angel JB and Kumar A. Down-regulation of CXCR-4 and CCR-5 expression by interferon-gamma is associated with inhibition of chemotaxis and human immunodeficiency virus (HIV) replication but not HIV entry into human monocytes. Clin Exp Immunol 2004;137:156-65

232. Kaufmann GR, Zaunders JJ, Cunningham P, et al. Rapid restoration of CD4 T cell subsets in subjects receiving antiretroviral therapy during primary HIV-1 infection. Aids 2000;14:2643-51

233. Goh LE, Perrin L, Hoen B, et al. Study protocol for the evaluation of the potential for durable viral suppression after quadruple HAART with or without HIV vaccination: the QUEST study. HIV Clin Trials 2001;2:438-44

234. Goh LE, McDade H, Kinloch S, et al. The QUEST trial, a paradigm of HIV collaborative research. Nat Med 2000;6:1194

235. Kinloch-de Loes S, Hoen B, Smith DE, et al. Impact of therapeutic immunization on HIV-1 viremia after discontinuation of antiretroviral therapy initiated during acute infection. J Infect Dis 2005;192:607-17

236. Tan SL, Katze MG. How hepatitis C virus counteracts the interferon response: the jury is still out on NS5A. Virology 2001;284:1-12

237. Atabani SF, Byrnes AA, Jaye A, et al. Natural measles causes prolonged suppression of interleukin-12 production. J Infect Dis 2001;184:1-9

238. Salek-Ardakani S, Arrand JR and Mackett M. Epstein-Barr virus encoded interleukin-10 inhibits HLA-class I, ICAM-1, and B7 expression on human monocytes: implications for immune evasion by EBV. Virology 2002;304:342-51

239. O'Brien SJ, Nelson GW. Human genes that limit AIDS. Nat Genet 2004;36:565-74

240. Betts MR, Nason MC, West SM, et al. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. Blood 2006;107:4781-9