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Leukemia Inhibitor Factor (LIF) and gp130 in early defence against HIV-1 infection

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Läsning avleder efter någon tid vår tanke alltför mycket från dess skapande uppgift. Varje människa som läser för mycket använder sin egen hjärna för litet och förfaller till lata tankevanor.

Albert Einstein

To Henrik, Elliott & Todd

Abstract

Leukemia inhibitor factor (LIF) is a polyfunctional cytokine that belongs to the IL-6 family which mainly signals through the Jak/Stat pathway via the gp130/LIFR-α heterodimer.

The focus of my research has been to investigate and understand if and how LIF exerts HIV-1 suppressing activity. We therefore examined the expression of LIF, and its receptors (gp130 and LIFR-α) in lymphoid tissue biopsies from primary HIV-1 infected (PHI), chronic HIV-1 infected (cHI) individuals and from long term non progressors (LTNP). Furthermore, consecutively obtained HIV-plasma samples collected from PHI individuals were analysed for LIF and soluble gp130 levels. Our data showed that LIF is one of the mediators of the innate immune response during HIV-1 infection. High production of LIF and gp130 were detected both in lymphoid tissue and in plasma from individuals with primary HIV-1 infection. Assesment of LIF plasma levels at PHI did not predict low levels of HIV-1 viremia after discontinuation of anti-retroviral treatment. However, a positive correlation between levels of plasma HIV-1 viral load and the production of LIF in lymphoid tissue or in plasma was found. In addition, a positive correlation between plasma levels of HIV-1 RNA and IFN-α, TNF-α, IL-1β, MIP-1α and MIP-1β were found in plasma from HIV-1 infected individuals that were in the primary phase of infection. After cessation of anti-retroviral treatment the levels of cytokines, including LIF, and chemokines were reduced as compared to the levels seen during primary HIV-1 infection. HIV-1 infected individuals that controlled their infection after cessation of treatment showed higher plasma levels of IFN-γ and MIP-1β as compared to individuals that did not control their HIV-1 infection. This suggests that during primary HIV-1 infection there is not a lack of a certain immune mediator that leads to immune failure, it is more like “too much and too many”. However, individuals that do control the infection appear to have a recall response to the virus, since they produce IFN-γ and MIP-1β which are suggested to be beneficial for the host to be abel to control the HIV-1 replication.

We also found that even though more than 50% of the total $CD4^+$ cells in lymphoid tissue expressed gp130, less than 5% of the total HIV-1 positive replicating cells ($p24^+$) in lymphoid tissue were $gp130^+$. Thus, LIF mediated a certain amount of control in $CD4^+gp130^+$ cells in lymphoid tissue. In addition, treatment of cMAGI cells with LIF prior to HIV-1 infection resulted in a dose dependent reduction in HIV-1 infected cells compared to untreated cells. Furthermore, both LIF and HIV-1 induced phosphorylation of Stat 3, and LIF pre-treatment resulted in a down modulation of the HIV-1 mediated Stat activation. Additionally, Jak/Stat inhibitors as well as siRNA against Stat 3 reduced HIV-1 replication. We suggest that the Jak/Stat pathway is important for HIV-1 replication and that LIF likely interferes with it.

In conclusion, LIF like many other cytokine and chemokines, is bifunctional since it has both HIV-1 suppressive action if present prior to HIV-1 infection, and HIV-1 enhancing activity if present after established HIV-1 infection.

Original papers

The thesis is based on the following original papers, which are referred to in the text by their Roman numerals:

I. **Annelie Tjernlund**, Zareefa Fleener, Homira Behbahani, Elizabeth Connick, Anders Sönnerborg, Christina Broström, Li-Ean Goh, Anna-Lena Spetz, Bruce K. Patterson and Jan Andersson: Suppression of leukaemia inhibitor factor (LIF) in lymphoid tissue in primary HIV-1 infection; absence of HIV-1 replication in gp130-positive cells. *AIDS* 2003, 17:1303-10.

II. **Annelie Tjernlund**, Babilonia Barqasho, Piotr Nowak, Sabine Kinloch, Daren Thorborn, Luc Perrin, Anders Sönnerborg, Lilian Walther–Jallow and Jan Andersson: Early induction of Leukemia inhibitor factor (LIF) in acute HIV-1 infection. *AIDS* 2006, **20**:11-19.

III. Babilonia Barqasho, Piotr Nowak, **Annelie Tjernlund**, Sabine Kinloch, Daren Thorborn, Luc Perrin, Jan Andersson and Anders Sönnerborg: Changes in plasma levels of cytokines and chemokines during primary HIV-1 infection and after cessation of antiretroviral therapy. *Manuscript*.

IV. **Annelie Tjernlund**, Lilian Walther–Jallow, Homira Behbahani Valentina Screpanti, Piotr Nowak, Alf Grandien, Jan Andersson and Bruce K. Patterson: Leukemia inhibitor factor (LIF) inhibits HIV-1 replication via restriction of Stat 3 activation. *AIDS Research and Human Retroviruses.* In press.

Contents

Abbreviations

Acquired Immunodeficiency Syndrome	AIDS
Adenosine Triphosphate	ATP
Antibody Dependent Cytotoxicity	ADCC
Antibody	Ab
Antigen Presenting Cells	APCs
Anti-Retroviral Treatment	ART
Apolipoprotein B mRNA-Editing Enzymes	APOBEC3G
Azidothymidine	AZT
Barrier to Auto Integration Factor	BAF
Cardiotrophin	CT
$CD8^+$ T lymphocyte Antiviral Factor	CAF
Chronic HIV-1 Infection	cHI
Ciliary Neurotropic Factor	CNTF
cMultinuclear Activation of Galactosidase Indicator cells	cMAGI cells
Complementary DNA	cDNA
Creb Binding Protein	CBP
Cycle Numbers	Ct
Cytotoxic T Lymphocytes	CTLs
Death Receptor 5	DR5
Dendritic Cells	DCs
Deoxyribonucleic Acid	DNA
Epstein Barr Virus	EBV
Gastrointestinal Tract	GT
Glycoprotein	gp
Granulocyte/Macrophage-Colony Stimulating Factor	GM-CSF
Heat Shock Proteins	HSP
High Mobility Group(I)Y	HMG(I)Y
Highly Active Anti-Retroviral Treatment	HAART
Highly Exposed Persistently Seronegative	HEPS
Human Immunodeficiency Virus	HIV
Hypothalamus-Pituitary-Adrenal	HPA
IL-1 Receptor Antagonist	IL-1Ra
Integrase	IN
Interferon Regulatory Factor	IRF
Interferon	IFN
Interleukin	IL
Just Another Kinase/Janus Kinase	Jak
Leukemia inhibitor Factor	LIF
LIF Diffusible	LIF-D
LIF Matrix-associated	LIF-M
LIF Receptor	LIFR
LIF Truncated	LIF-T
Long Term Non-Progressors	LTNPs
Long Terminal Repeats	LTRs
Lymphoid Tissue	LT
Macrophage Inflammatory Protein	MIP
Major Histocompatibility Complex	MHC
Mannose Binding Lectins	MBL
Matrix	MA
Messenger RNA	mRNA
Myleoid DCs	mDCs
Natural Killer cells	NK cells
Nuclear Factor Kappa B	NF-κB
Oncostatin M	OSM
Pattern Recognition Receptors	PRRs
Peripheral Blood Mononuclear cells	PBMCs
Plasmacytoid DCs	pDCs
Poly protein Processed Protein	PR protein

Polymerase Chain Reaction	PCR
Pre-Integration Complexes	PICs
Primary HIV-1 Infection	PHI
Protein Inhibitor of Activated Stats	PIAS
Regulated on Activation, Normal T cells Expressed and Secreted	RANTES
Reverse Transcriptase	RT
Reverse Transcription PCR	RT-PCR
Ribonucleic Acid	RNA
Signal Transducer and Activator of Transcription	Stat
Simian Immunodeficiency Virus	SIV
Small Interfering RNA	siRNA
Soluble gp130	sgp130
Stromal Cell-Derived Factor-1	SDF-1
Suppressor Of Cytokine-Signaling	SOCS
TNF-Related Apoptosis-Inducing Ligand	TRAIL
Toll-like Receptors	TLRs
Transactivation Response Elements	TAR
Tuberculosis	TB
Tumor Necrosis Factor	TNF

Human Immunodeficiency virus

General introduction to HIV-1 infection

The first medical reports which led to the definition of acquired immunodeficiency syndrome (AIDS) emerged in 1981 in California and in New York City when a number of young homosexual men displayed symptoms of rare diseases normally associated with severely immuno-compromised patients[1-3]. The causative agent, Human Immunodeficiency virus (HIV) was identified by Dr. Luc Montangier at Pasteur Institute in Paris and later by Dr. Robert Gallo at the National Institutes of Health, USA[4, 5] during 1983. Another form of HIV, HIV-2, was identified 1986 by Clavel *et al*[6]. These viruses were found to be spread by sexual contact, by parenteral contact including blood, and by vertical transmission from mother to child.

Phylogenetic evidence showed that HIV originates from the Simian Immunodefiency virus (SIV). HIV-1 arose from SIVcpz (chimpanzees) and HIV-2 from SIVsm (sooty mangabeys), respectively[7-10]. The virus that is responsible for the current HIV-1 epidemic has been estimated to have entered the human population between 1915-1940 in Central Africa[11]. Until today, there have been nine HIV-1 subtypes (A,B,C,D,F,G,H,J,K) and several “circulating recombinant forms” described[12]. The HIV-2 groups recognized are A-H[13].

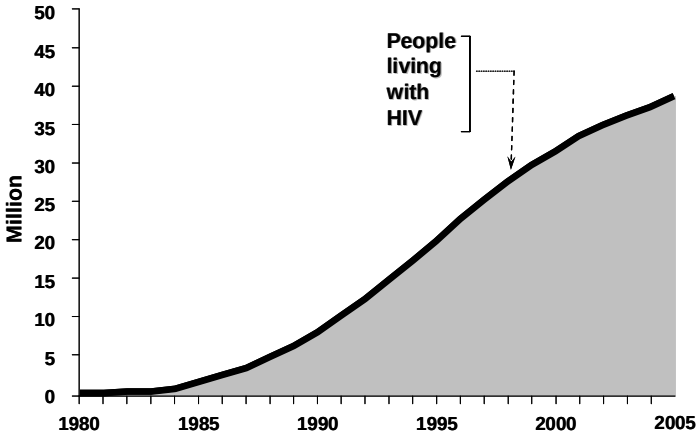

Figure 1. *The graph illustrates how the numbers of HIV-1 infected individuals have risen from the onset of the epidemic until year 2005. Adapted from UNAIDS, 2006.*

HIV has caused one of the most devastating epidemics in recorded history. It is estimated that more than 60 million people have been infected with HIV worldwide and that more than 25 million had died of AIDS by end of 2005

(Figure 1)[14]. HIV/AIDS is the leading cause of deaths and loss of productive life years in adults aged 15-59. There were approximately 5 million people who became infected and 3 million people who died due to HIV/AIDS only during year 2005[14].

Properties of the virus

HIV-1 is a double stranded RNA Lentivirus which belongs to the Retroviridae family[15]. The viral particle is 100 nm in diameter and has a 9.2 kb long genome which contains three major structural genes; *gag*, *pol* and *env* that are flanked on both sides by long terminal repeats (LTRs), which are the regions that are connected to the cellular DNA of the host cell after integration, and serves as the promoter region for transcription of the virus.

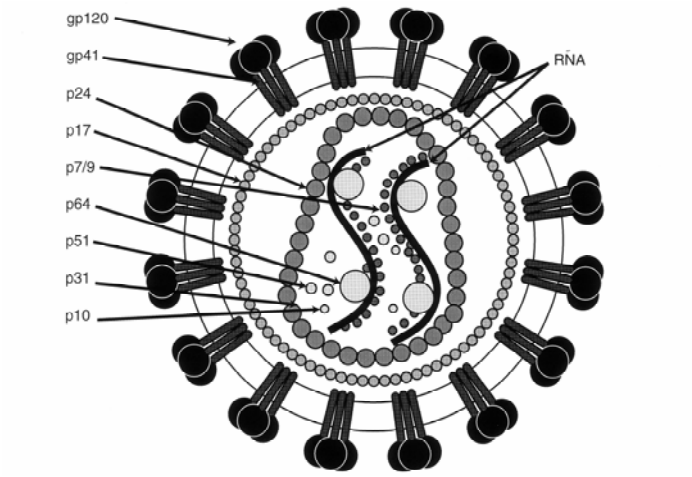

Figure 2. *The molecular structure of HIV-1.*

The *gag* gene encodes the structural proteins; capsid (p24), matrix (MA, p17) and nucleocapsid (p9 and p7). The *pol* gene encodes reverse transcriptase (RT), integrase (IN) and polyprotein processed (PR) protein. These proteins are essential enzymes for transcription of viral RNA into DNA, integration of viral DNA into the human genome and cleavage of HIV-1 proteins, respectively. The *env* gene codes for the envelope glycoproteins gp120 and gp41 which are important for virus binding and subsequent infection of target cells. In addition, the HIV-1 provirus consists of six additional open reading frames which code for the regulatory proteins (Rev and Tat) and for the accessory proteins (Vif, Vpu, Nef and Vpr). Rev and Tat accumulate within the nucleus and bind to defined regions of the viral RNA. Rev is involved in regulation of viral gene expression, it binds to Rev response elements (RRE) that are found in the *env* gene, whereas Tat is involved in activating transcription; it binds to the transactivation response

elements (TAR) within the LTR. The accessory proteins are thought of as auxiliary proteins since they are not needed for viral production although they facilitate it. Furthermore, they are important targets for the immune response against HIV-1 (discussed later).

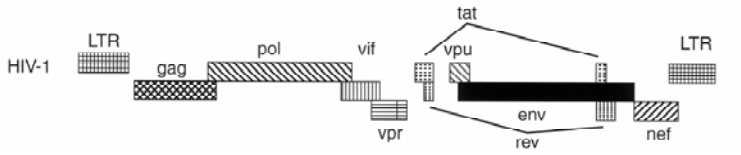

Figure 3. *The genetic structure of HIV-1.*

The viral life cycle

A dynamic interaction between the virus and the host can be observed at every step of the life cycle of HIV-1. Host factors are involved not only in anti-viral responses but are also hi-jacked by the virus to perform pro-viral functions or are suppressed by the virus to minimize their potential anti-viral functions[16]. The life cycle of HIV-1 (Figure 2) starts with HIV-1 entry into target cells, that is mediated by the trimeric form of the env spike glycoproteins, gp120 and gp41, on the mature infectious virus particle. gp120 interacts with the appropriate receptor (CD4) on the cell surface[17, 18]. This leads to a conformational change that enables gp120 to bind to its co-receptor, usually CCR5 (for CCR5-using virus) or CXCR4 (for CXCR4-using virus) in combination with CD4[19-23] and this subsequently results in a conformational change of gp41 into a fusogenic state[24]. Fusion of the virus with the host cell is thought to take place in a pH-independent manner. The co-receptors are contained within lipid rafts, cellular microdomains that are rich in cholesterol, which provide a supportive environment for viral fusion and entry since the lipid rafts are of similar composition as the lipid bi-layer of the virion envelope[25]. Apolipo protein B mRNA-editing enzyme, catalytic polypeptide-like 3G (APOBEC3G) is a cytidine deaminase (it converts cytosines to urasils) which has been shown to have antiviral activities in the early post-entry steps[26, 27]. However, if the HIV-1 protein Vif is present, it binds to ABOBEC3G and targets it for degradation through the cellular proteasomes. TRIM5-α is a restriction factor which blocks the early replication of retroviruses by preventing the accumulation of reverse transcription products[28]. The next step for the virus is to shed its nucleo-capsid and release the viral nucleoprotein machinery into the host cell so that the viral

enzyme RT can catalyze the reverse transcription of viral RNA into cDNA molecules which thereafter are assembled into pre-integration complexes (PICs)[29, 30]. These large nucleoprotein complexes contain, in addition to viral nucleic acid, Intergrase (IN), Reverse transcriptase (RT) and Matrix (MA) proteins among other yet unidentified proteins. Phosphorylation of the MA protein promotes translocation of the reverse transcriptase complexes from the membrane to the cytoplasm and subsequently to the nucleus[31]. Lentiviruses, including HIV-1, have developed means to actively transport PICs through nuclear membranes which makes it possible to infect non-dividing cells (oncoretroviruses can only gain access to the host genome when the target cells undergo mitosis)[16, 32, 33]. The PICs are integrated to the human genome with help of the viral enzyme IN and is assisted by several host factors[34, 35].

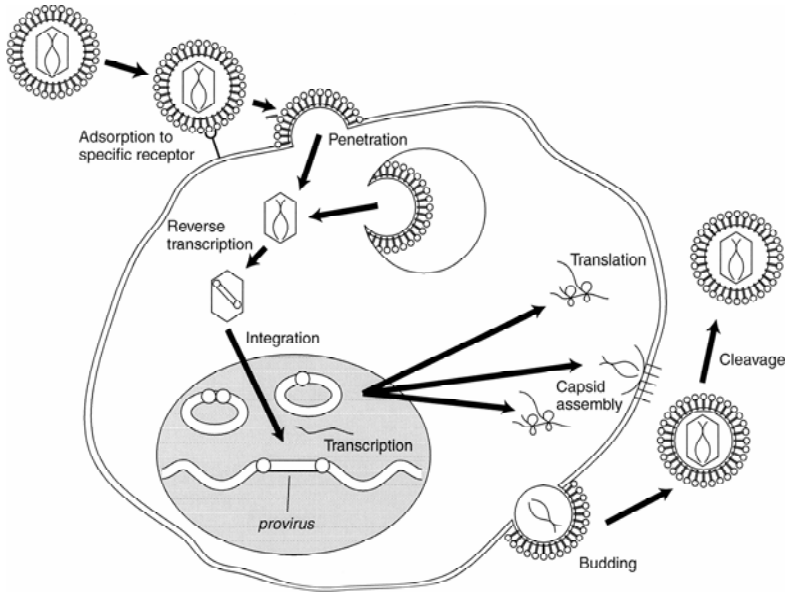

Figure 4. *The HIV-1 life cycle. The virus infects a susceptible cell by binding to CD4 and a co-receptor (usually CCR5) which results in fusion with the cell membrane. The viral genome is reverse transcribed and integrated to the host genome (provirus). This is followed by transcription and translation of the viral genome which results in new viral particles that are assembled in close vicinity to the cell membrane were the fully mature virions bud off.*

Transcription of the integrated viral DNA, called provirus, is a crucial step in the viral lifecycle that involves a highly regulated interplay between the virus and the host machinery. A key cellular transcription factor involved in HIV-1 replication is nuclear factor (NF)-κB[36]. After stimulation with mitogens or cytokines, NF-κB is activated in the target host cell and is translocated to the nucleus where it binds to the specific target sequence of the LTR and activates HIV-1 transcription. Transcription initially results in the synthesis of Tat and Rev. Tat enhances HIV-1 gene expression by binding directly to the TAR region of the LTR with the help

of CyclinT1, an essential cellular co-factor. CyclinT1 associates with Tat and this results in recruitment of the CDK9 kinase to the TAR sequence[37-39]. This subsequently leads to the formation of a transcription elongation complex that in turn results in hyper-phosphorylation of the C-terminus of RNA polymerase II needed to get efficient elongation of nascent RNA molecules. Another protein that Tat also interacts with in order to enhance transcription is the cellular transcription factor Sp1 which can act as an antagonist to Sp3 that is known to repress LTR driven transcription in certain cells[39, 40]. Co-activator proteins such as Creb binding protein (CBP)/p300 and pCAF are also involved in the Tat-induced transcriptional activation of the LTR promoter through the remodeling of the HIV-1 containing chromatin structure[41]. Furthermore, they mediate acetylation of Tat and thus promote its transcription activity. The HIV-1 protein Rev is important for switching from the early expression of regulatory proteins to expression of structural proteins thereby promoting the formation of mature viral particles. The *pol* and *gag* genes code for precursor proteins which after cleavage by the viral enzyme protease, form the nucleus of the mature HIV-1 particle. The cytoskeleton directs the transport of viral complexes to the inner membrane of the cells. The virus assembles within cholesterol-rich lipids rafts at the surface of the host cell and new virions bud of from the cell membrane. During the budding process, the virus lipid membranes may incorporate various host cell proteins such as chemokine receptors that facilitate fusion with the next target cell [42]. The HIV-1 protein Vpu is important for the viral budding process[43].

HIV-1 pathogenesis

In general, HIV-1 infection follows three phases (Figure 5). The acute or primary phase lasts for about two to three months and it is probably the most dynamic phase[44]. In the first hours or days of infection, virus or infected cells, cross the mucosal barrier (if infection occurs through sexual transmission)[45-47]. A local infection becomes established at the point of entry if the virus is “successful”. Factors such as; low pH, hydrogen peroxide, innate factors (discussed later), entrapment of infected cells and/or virus in the mucus that overlies the epithelium limits the chance for the virus to reach its targets cells[48, 49]. Furthermore, the physical barrier of the epithelial lining is crucial since

destruction of the barrier integrity will enhance the rate of transmission significantly [50-54]

The first cells that become infected are “resting” memory $CD4^+$ T cells mainly due to the fact that they are in the right place at the right time (or perhaps more correctly; in the wrong place at the wrong time)[47, 55-57]. Other target cells for the virus are activated $CD4^+$ T-cells, Macrophages and Dendritic cells (DC) since they all express the main receptors, CD4 and CCR5 or CXCR4, that HIV-1 need in order to infect cells[47, 58-60]. It is mainly CCR5-using isolates that are involved in the establishment of primary infection since the cells that are present in the submucosa primarily express CCR5[61-63]. CXCR4-using isolates usually emerge later in disease progression and are suggested to be more aggressive types of virus that is associated with a faster progression to AIDS[64, 65].

The HIV-1 infected cells start to produce and shed viral particles and transmit the virus laterally to neighbouring targets. Infected cells and virus carried by DCs will also spread to draining lymph nodes and then through the blood stream to peripheral lymph nodes, the gastrointestinal (GI) tract and the spleen. At approximately one week after exposure, viral RNA can be found systemically[66-69]. The milieu in lymphoid tissues (LT); large numbers of susceptible cells in close proximity, makes it favorable for the virus to increase to high number and to spread. During the acute phase of HIV-1 infection the numbers of $CD4^+$ T cells declines rapidly and even though it rebounds, with resolution of the acute phase, it rarely returns to baseline levels in the absence of anti-retroviral treatment (ART). It is specifically memory $CD4^+$ T cells that are lost and in particular those in the GI tract[55, 70]. The vast majority of T cells in the GI tract express CD95 (Fas), and are therefore prone to undergo apoptosis if targeted by its ligand, the cell associated trimeric Fas ligand (CD95L). This is thought to be a consequence of a normal mechanism to induce tolerance in order not to get pathological gut inflammation since the T cells in the GI tract are exposed to high doses of antigen daily[71, 72]. High concentration of viral gp120 triggers CD95L expression on the target cells, and binding of trimeric CD95L to CD95 induces apoptosis[73]. This may even occur within the same cell. In addition, induction of INF-α leads to upregulation of expression of death molecules, such as TNF-related apoptosis-inducing ligand (TRAIL) and death receptor (DR)5. The TRAIL/ DR5 pathway contributes to the apoptosis of both infected and

uninfected cells[74-78]. Of course the virus will also lyse infected cells due to its lytic cycle. The total outcome is a huge depletion of $CD4^+$ T cells within a few days, which results in "substrate exhaustion" that is thought to partly be responsible for the gradual decline in viral load during HIV-1 infection[79, 80]. The specific immune response; neutralizing antibodies against HIV-1, HIV-1 specific $CD8^+$ cytotoxic T lymphocytes (CTL) and HIV-1 specific $CD4^+$ helper cells (all discussed later) are also thought to be responsible for the decline of the viral load to steady state[81, 82]. This viral set point is associated with disease outcome[66, 83]. High viral load is associated with a rapid progression and a low viral load correlates with better disease prognosis. The primary infection phase can be asymptomatic, but usually 50% of infected individuals exhibit fever, oral ulcers, maculopapular rash, sore throat, headache and swollen lymph nodes, resembling the symptoms of mononucleosis[84, 85].

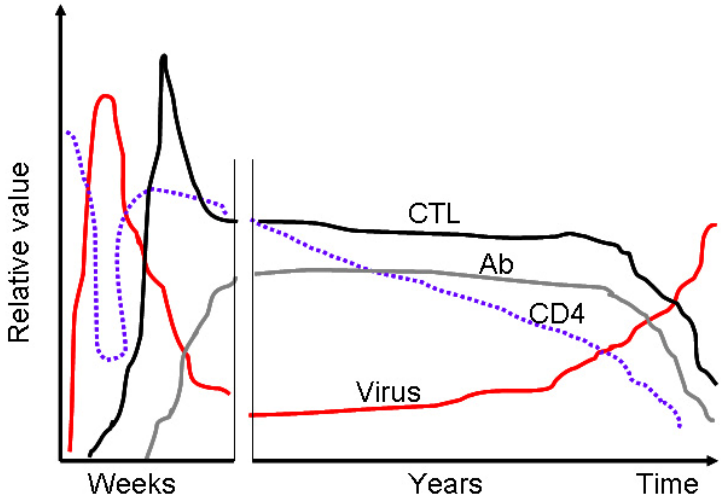

Figure 5. *Schematic illustration of the natural course of a typical HIV-1 infection showing viral load (red line), $CD4^+$ T cell counts (purple dotted line), CTL reponse (black line) and Ab response (grey line).*

The chronic stage is usually an asymptomatic phase that can last for many years[86-88]. The viral load is kept low even though there is a persistent viral replication going on in the peripheral lymphoid tissues[67, 89, 90]. The gradual loss of $CD4^+$ T cells will eventually lead to dysfunction of the immune system and the appearance of opportunistic infections and malignancies that are associated with AIDS, the last and final stage of HIV-1 infection. 200 $CD4^+$ T cells/μl blood (representing approximately 80% loss of circulating $CD4^+$ T cells) is an important cut-off level since below that the appearance of AIDS defining illnesses (opportunistic infections, neoplasm and malignancies) occur[91].

There are a few percent of HIV-1 infected individuals which preserve immune function and have normal $CD4^+$ T cell numbers for more that 10-15 years. These are termed long-term non-progressors (LTNP).

Innate immune response

Innate immunity is the first line of defence against invading pathogens. It is rapid, (acts within minutes to hours), is found at mucosal surfaces (the major entry point for HIV-1), has no memory and recognize “microbial non-self” by pattern recognition receptors (PRRs) which bind to highly conserved structures on pathogens. The innate immune response can also detect “altered self” or “missing self” that will lead to phagocytosis or lysis, respectively, of the cell/pathogen in question[92]. The innate immune system includes a number of different cell types; DC, natural killer (NK) cells, γδT cells, macrophages, monocytes and neutrophils and of soluble components; mannose-binding lectin (MBL), proteins of the complement system, cytokines, chemokines and defensins. They are all important players in the innate immune response with capacity to eradicate or at least limit the spread of pathogens and also to provide the stimuli and the time needed for the adaptive immune response to develop[93].

Innate immune response against HIV-1

Cells

Immature DCs are sentinels residing in peripheral tissues that capture antigen; they are good phagocytes and express a variety of PRRs, including Toll-like receptors (TLRs)[94]. Upon antigen uptake and/or signalling via PRRs the DCs mature to professional antigen presenting cells (APCs) and migrate to LT where they primarily interact naïve T cells and provide the stimulus that the T cells need in order to become fully mature effector cells. There are two main subsets of DCs, myeloid DCs (mDCs) and plasmacytoid DCs (pDCs). mDCs are the main producers of IL-12 that are important to induce cell mediated Th1 like immune response[95, 96] whereas pDCs are the main producers of IFN-α and IFN-β, which among many other effects can inhibit HIV-1 replication and activate NK cells[97-99]. Thus, DCs are key mediators in the innate immune response and an important link between the innate and adaptive immune response against

pathogens, including HIV-1[92, 100]. As discussed above, like CD4 T cells, DCs express all the major HIV-1 receptors (CD4, CCR5 and CXCR4) and they can therefore become infected by HIV-1[101-105]. In addition, HIV-1 can also bind to the cell surface of DCs through binding to the DC-SIGN receptor or other lectin receptors and be internalized[59, 106, 107]. Within the lymphoid compartment DCs can form conjugates with adjacent T cells and infectious synapses are formed which results in very efficient viral spread[108]. It is reported that during HIV-1 infection there is a reduction in numbers of DCs in the blood, mainly in those individuals with active viral replication[109-112]. Numerous studies have observed that their function is impaired; DCs migrate to LT during acute HIV-1 infection, they express CD40 but the co-stimulatory molecules, CD80 and CD86, are only partially upregulated[113] and HIV-1 infected DCs to not produce IL-12[114]. HIV-1 infected DCs do not undergo maturation, but secrete IL-10 which subsequently can lead to T-cell anergy[115]. The importance of DCs in HIV-1 infection can be seen in LTNP which have been reported to have increased numbers of functional pDC as compared to progressors and uninfected controls[116].

NK cells have the capacity to release several molecules that are involved in antiviral defense (INF-γ, TNF-α, MIP-1α, MIP-1β, RANTES, perforin and granzymes)[117]. NK cells can kill virus infected cells directly or by antibody dependent cellular cytotoxicity (ADCC). They are therefore considered to be the main effector cells of innate immunity. NK cells and DCs have been suggested to, in part, be responsible for protection against HIV-1 infection in highly exposed seronegative individuals (HEPS)[118]. Furthermore, NK cell function also seems to be impaired in HIV-1 infected individuals since both reduced secretion of β-chemokines and reduced cytotoxic activity due to downregulation of activating receptors on the surface of NK cells have been reported[119-121].

Other cells that play a role in the innate immune response against HIV-1 infection are $\gamma\delta$T cells which are able to lyse virus infected cells and produce β-chemokines[122]. Furthermore, the phagocytic oxidative capacity of macrophages[123] and the superoxide production by neutrophils have also been reported to be impaired in HIV-1 infected individuals[124].

Innate soluble factors

The $CD8^+$ T-lymphocyte anti-viral factor (CAF), which has not yet been identified, is active against HIV-1, HIV-2 as well as SIV[125-127]. One part of CAF action is inhibition of HIV-1 RNA transcription, particularly during LTR-driven gene expression. Chang *et al* have showed that Stat 1 is necessary for CAF-mediated inhibition of LTR activation and HIV-1 replication. Furthermore, CAF induced the expression of interferon regulatory factor 1 (IRF-1), and IRF-1 gene induction was shown to be Stat 1 dependent, suggesting that CAF activates Stat 1, leading to IRF-1 induction and inhibition of gene expression regulated by the HIV-1 LTR[128].

MBL and proteins from the complement system have been shown to bind to HIV-1 and lyse it directly or work as an oppsonizer thus facilitating viral phagocytosis by macrophages[129-132].

HIV-1 infection and replication is influenced by many different soluble factors and their effect on HIV-1 can either be stimulatory, inhibitory or both (bifunctional). The latter is particularly true for the action of cytokines and chemokines which normally form a complex network that is thought to be dysfunctional during HIV-1 infection. IFN-α and IFN-β are produced primarily by monocytes and pDC. They enhance the cytotoxicity of T cells and NK cells and inhibit regulatory T cells[133]. Furthermore, IFN-α is a potent anti-HIV-1 molecule which inhibits multiple steps of the HIV-1 life cycle[134-136] and has been reported to upregulate expression of the anti-viral factor APOBEC3G[137]. IFN-γ is produced by activated T cells and NK cells and is a bifunctional cytokine. It has been shown to inhibit HIV-1 via down regulation of CD4 or alternatively by activation of the Jak/Stat signalling pathway[138-141]. Studies have also shown that IFN-γ activates HIV-1 transcription primarily by synergistically potentiate the stimulatory effects of TNF-α[142-144]. IL-12 is produced by macrophages and DCs. It stimulates T cells and NK cells to produce IFN-γ and thus polarizes the immune systems towards a Th1 like response. It has also been reported that IL-12 is bimodal; it induces HIV-1 replication[145-147] and inhibits HIV-1 replication if the cells are pre-treated with IL-12, which is suggested to be due to downregulation of CCR5[148, 149]. IL-15, like IL-12 are produced by macrophages and DCs and enhances replication of HIV-1[145, 146]. Proinflammatory cytokines,

such as TNF-α (produced by macrophages, NK cells and T cells), IL-1α and IL-1 β (produced by macrophages and epithelial cells), IL-6 (produced by T cells, marophages and epithelia cells) and IL-18 (produced by macrophages) are thought to enhance HIV-1 replication through NF-κB activation and upregulation of CCR5 expression[150-159]. LIF (discussed later) is produced by a variety of cells and is a polyfunctional cytokine that belongs to the IL-6 family[160].

Chemokines are produced by DCs, Macrophages, monocytes, NK cells, γδT cells and activated T cells[161]. α-chemokines (SDF-1) are natural ligands for CXCR4 while β-chemokines (MIP-1α, MIP-1β, RANTES and eotaxin) are natural ligands for CCR5 and have therefore been suggested to compete with HIV-1 by binding to CXCR4 and CCR5, respectively, and either block HIV-1 or down regulate the receptors[22, 162-165]. However, chemokines may also mobilize $CD4^+CXCR4^+$ and $CD4^+CCR5^+$cells and thereby increase HIV-1 infection rate.

Defensins are small cationic peptides with anti-microbial activity that serve as important effectors in innate immunity against HIV-1[166, 167]. So far, six α-defensins and 28 β-defensins have been identified (six have been characterized) in humans and three theta-defensins have been identified in rhesus macaques. Both α- and β-defensins exhibit chemotatic activity for T cells, monocytes and immature DCs and induce cytokine production[168-170]. Alpha-defensins are mainly found in neutrophils but also in NK cells, B cells, γδT cells, monocytes, macrophages and epithelial cells[171, 172]. Alpha-defensins inactivate HIV-1 replication in at least two ways; directly by binding to the virus and inactivating it in a serum free milieu like the mucosal surface, and indirectly by acting on the target cell by blocking HIV-1 infection at the step of nuclear import and replication[169, 173, 174]. β-defensins produced by epithelial cells are suggested to be part of the salivary components (mucin, amylase and SLP-1 etc) that play an important role in the prevention of HIV-1 transmission in the oral cavity[175-177]. Quinos-Mateu *et al* have showed that both CXCR4-using and CCR5-using viruses induces secretion of β-defensins 2 and 3 from human oral epithelia[178]. Furthermore β-defensins 2 and 3 inhibit viral replication preferentially of CXCR4-using viruses by acting as antagonists[179]. Theta-defensins from rhesus bind carbohydrate epitopes displayed on viral particles (gp120) and cell-surface

glycoproteins (CD4) that are involved in viral entry thereby inhibiting HIV-1 viral replication [170, 180-182].

Adaptive immune response against HIV-1

The adaptive immune response, in contrast to the innate immune response, is "slow", has memory and can provide specific recognition of foreign antigens in a MHC-restricted fashion. HIV-1 infection results in generation of virus-specific antibodies, HIV-1 specific T-cells (CD4 helper cells, CTL, and regulatory T cells) as well as production of cytokines and chemokines.

Antibody (Ab) responses develop early in HIV-1 infection and the major targets for neutralizing Abs are the envelope proteins gp120 and the external part of gp41[183-185]. HIV-1 mutates and replicates at a high rate and this in combination with the fact that the gp120 is heavily glycosylated, and thus poorly immunogenetic, makes it hard to achieve a good neutralizing Ab response. The impairment of HIV-1 specific $CD4^+$ T cell function occurs early during the acute phase[186, 187] and is probably due to fact that it is preferentially HIV-1 specific $CD4^+$ T cells that are infected with HIV-1[56, 188].These cells are killed by virus induced lysis, by HIV-1 specific CTL, or by ADCC resulting in a huge depletion of cells. Several reports demonstrate the importance of HIV-1 specific $CD8^+$ T cells during the course of infection[189-198]. Recently it has been suggested that HIV-1 specific $CD8^+$ T cell responses that are polyfunctional correlate inversely with viral load[199]. However, the function of the majority of $CD8^+$ T cells in HIV-1 infected individuals is impaired since skewed maturation of HIV-1 specific memory $CD8^+$ T cells[200], down modulation of the CD3 zeta chain[201], lack of perforin expression[202-205] and accumulation of regulatory T cells in LT[206] has been reported. Furthermore, both HIV-1 and SIV have been shown to induce mutations in the genes encoding for amino acid residues important for $CD8^+$ T cell recognition subsequently leading to viral escape [198, 207-210].

Anti-retroviral treatment

In 1987 the first anti-retroviral drug, a nucleoside analogue called azidothymidine (AZT) was approved. During 1995 and 1996 two new classes of antiretroviral drugs, non-nucleoside analogues and protease inhibitors were introduced. The combinations of these drugs were called highly active anti-retroviral treatment (HAART) and were shown to be very efficient in reducing the

HIV-1 load and to keep viral resistance low[211, 212]. At first there was hope for virus eradication, however, many studies have shown that discontinuation of anti-retroviral treatment (ART) led to rapid viral rebound in most infected individuals. It has been estimated that more than 70 years of anti-retroviral treatment (ART) would be required for virus eradication since latently infected cells such as inactive $CD4^+$ T cells, monocytes, macrophages and microglia cells serves as reservoirs for HIV-1[213-218].

Today, there are three classes of anti-retroviral drugs; transcriptase inhibitors, protease inhibitors and entry inhibitors. There are also new drugs in the pipeline which are small molecule inhibitors that target IN and RT which hopefully will make treatment even more promising[219].

Why is HIV-1 infection not cleared?

As discussed above, HIV-1 infection results in a huge depletion of $CD4^+$ T cells and impairment of both $CD4^+$ and $CD8^+$ T cell function that subsequently results in a dysfunctional immune system. On top of this, HIV-1 can not only infect activated/dividing cells but also non-dividing cells. HIV-1 thus, does not only destroy the immune system but can also hide from it. Furthermore, ART is unable to affect non-replicating pro-viruses since the drugs today targets the event of fusion, the reverse transcriptase enzyme, and the protease enzyme but does not target latent proviruses. There is great need for treatment that results in immune reconstitution and drugs that target alternative steps within the life cycle of HIV-1.

What should a successful vaccine look like?

An optimal vaccine against HIV should stimulate the innate immunity, generate broadly cross-reactive neutralizing Ab and also induce HIV specific polyfunctional CTLs. This should, preferentially, occur not only systemically but also at local sites (the mucosa). Furthermore, the vaccine should be safe and be available globally. This is a daunting challenge! Despite initial optimism and evaluation of more that 30 products in more than 85 trials, the search for an HIV vaccine has yet not reached its goal[220]. Most ongoing trials are testing vaccine candidates that are meant to induce HIV-specific cellular immunity. This is achieved by using viral vectors (expressing HIV proteins), either alone or in combination with DNA used in so called prime boost strategies. Studies in

experimental SIV infected monkeys have showed that the use of new vaccine adjuvants, such as unmethylated CpG motifs, heat shock proteins (HSP) and GM-CSF, results in innate immune activation; production of HIV/SIV-inhibiting cytokines and chemokines which are proposed to be important in modulating and steering the adaptive immune response[221].

Microbicides

The term microbicides refers to a broad range of products whose common function is to prevent infection by HIV and other sexually transmitted pathogens when applied topically in the vagina or rectum. There are several microbicides that are in clinical trials and they act by killing or inactivating pathogens; by creating physical barriers (surfactants, gels or creams), by strengthening the body's normal defences (pH regulators like the natural occurring Lactobacillis bacterium), by inhibiting viral entry (anti-HIV glycans, fusion inhibitors and CCR5-based inhibitors) and by inhibiting viral replication (nucleoside transcriptase inhibitors and non nucleoside transcriptase inhibitors. [222]. Microbicides will hopefully be a cost-beneficial prophylaxis available for people in developing countries.

Leukemia Inhibitor Factor

Leukemia Inhibitor Factor (LIF) is a glycoprotein with an approximate molecular weight of 20 kDa which consists of a single four α-helix polypeptide chain [223]. LIF was cloned and characterized by Dr. Donald Metcalfs group during 1987-88 and was identified as a factor able to induce macrophage maturation and terminate self-renewal of the undifferentiated and highly clonogeneic murine myeloid leukemia, M1[224-227]. The combination of these actions suppressed the leukemic population, hence the name assigned.

The LIF gene has three alternative first exons which can be spliced to common second and third exons, yielding three transcripts. These transcripts are regulated independently by using different promoters. The three transcripts are translated into three different protein forms of LIF; LIF-D, LIF-M and LIF-T[228-230]. The LIF-D (diffusible) transcript encodes for an extracellular protein that is freely diffusible, the LIF-M (matrix-associated) transcript encodes for a protein which can be found intracellularly and can be secreted as an extracellular matrix

associated protein. The LIF-T (truncated) transcript encodes for a intracellular protein that can not be secreted since the initiation of protein translation is downstream of the secretion signaling sequence. Differential promoter usage coupled with alternate splicing has also been shown to regulate production of intracellular and extracellular localized human IL-15 and IL-1Ra[231-233].

LIF is a polyfunctional cytokine that has a wide array of actions; it is important for hematopoesis since it acts as a hematopoietic growth factor and it is also used extensively in experimental biology because of its key ability to induce embryonic stem cells to retain their totipotentiality[234-238]. In addition, studies in mice have suggested the importance of LIF in successful pregnancy outcome [239, 240] and studies in humans have shown that endometrium of infertile women produces significantly less LIF during the period of receptivity. However, the role of LIF in unexplained infertility and implantation failures in humans is not clear[241, 242]. Furthermore, LIF has potent pro-inflammatory properties since LIF induces synthesis of acute phase proteins and affects cell recruitment into the area of damage or inflammation[243]. LIF is expressed in many different tissues and is upregulated after stimulation with IL-1 and TNF-α[244-246]. Elevated levels of LIF has been reported in a variety of inflammatory conditions such as, bacterial infections, auto immune diseases, and different forms of cancer [246-248]. It is proposed that LIF may function as an initial signal for host inflammatory cytokine production. In addition to its pro-inflammatory activity, LIF also plays a key role in the hypothalamus-pituitary-adrenal (HPA)-mediated stress response[249]. Thus, by inducing corticosteroid synthesis it also has anti-inflammatory properties. Perhaps due to its many different functions, LIF has been used in different clinical studies and is proven to be relatively nontoxic[250, 251].

LIF signaling

LIF belongs to the IL-6 cytokine family which consists of IL-6, IL-11, oncostatin M (OSM), ciliary neurotropic factor (CNTF) and cardiotrophin (CT-1)[160]. All members of the IL-6 cytokine family signal through the common gp130 receptor subunit[252]. In addition, each member also has a specific receptor. In the case of LIF, it is LIF receptor-α (LIFR-α) which LIF binds to with low affinity. Thereafter the LIF/LIFR-α-complex associate with the gp130 receptor and a high affinity complex is formed, which is needed in order to activate the Jak/Stat signaling

pathway (Figure 6). The Jak/Stat signaling pathway was originally identified as an interferon-activated transcription factor by Darnell and colleagues[253]. Today, it is known to be used by many different mitogens and cytokines.

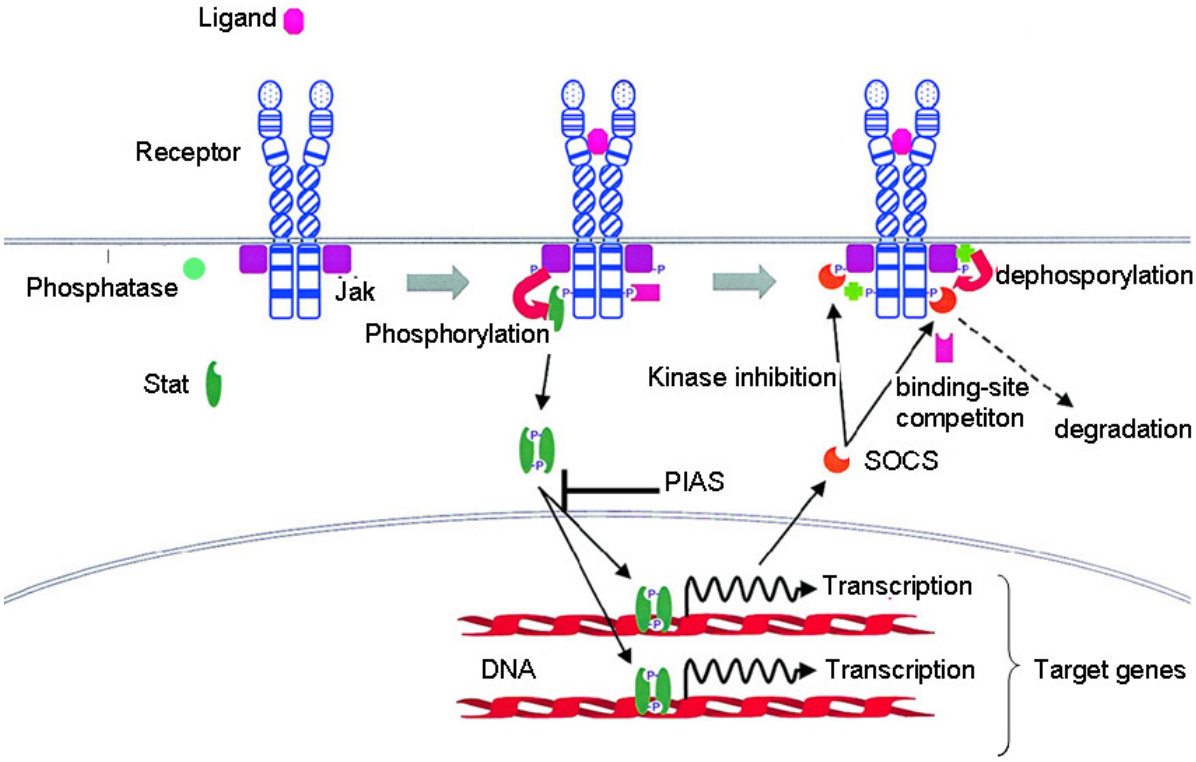

Figure 6. *Schematic picture of the Jak/Stat signaling pathway. A ligand (for example LIF) binds to its receptor (LIFR-α and gp130) which results in dimerization of the receptors and phosphorylation of Jaks followed by phosphorylation, dimerization and translocation of Stat proteins. Within the nucleus Stat proteins binds to specific recognition sites within the genome and transcription of target genes occurs. The Stat signaling pathway is negatively regulated by SOCS and PIAS. Adapted from Ward AC, Blood 2000.*

Since gp130 lack intrinsic kinase activity it is dependent upon cytoplasmic Janus kinases (Jaks) which are associated with the cytoplasmic part of gp130 in the absence of ligand. Binding of the ligand causes conformational changes and heterodimerization of gp130/LIFR-α which results in auto-phosphorylation and activation of Jaks. Phosphorylated tyrosine residues on gp130 and LIFR-α serve as specific docking sites for the SH2-domaines of signal transducers and activation transcription (Stat) proteins. This subsequently results in phosphosrylation of Stat proteins followed by dimerization and translocation of the Stat proteins to the nucleus and expression of genes with Stat recognition sites. LIF-induced Jak/Stat signaling cascade is negatively regulated by both suppressor-of cytokine-signalling proteins (SOCS), which inhibits Jak activity, and by protein inhibitors of activated Stat (PIAS) that interact with activated Stat and inhibit their binding to specific DNA sequences[254]. LIF mainly activates Stat

1 and 3 and the pattern of Jak/Stat protein activation is cell-type specific. Additionally, LIF has also been reported to activate the Ras/MAPK pathway[252, 255].

LIF and HIV-1 infection

The first report concerning LIF and HIV-1 infection came in 1994 when Broor *et al* showed that LIF induced a dose-dependent increase of p24 antigen production in the chronically infected promonocytic cell line U1[256]. The magnitude and time kinetics of the LIF mediated effects were similar to IL-1, IL-6, and TNF, other cytokines known to induce HIV replication in this cell line. They showed that LIF increased levels of HIV mRNA and they proposed that it was due to increased activation of the DNA binding protein nuclear factor (NF)-*k*B which plays a major role in the regulation of HIV-1 gene expression[36].

In 2001 Patterson *et al* showed that expression of placenta derived LIF was associated with protection against vertical transmission of HIV-1 from mother to child[257]. Pre-treatment with LIF inhibited HIV-1 replication *in vitro* in peripheral blood mononuclear cells (PBMCs) as well as in placenta and thymus organotypic explant models. This inhibitory effect seen when LIF was present prior to HIV-1 exposure occurred with at least a 100-fold lower concentration of LIF than the inhibitory concentration needed to induce a similar effect with ß-chemokines (RANTES and MIP-1 α and β)[165]. The LIF-mediated anti-HIV-1 activity occurred prior to reverse transcription and was dependent upon cell-surface expression of the signaling receptor gp130.

Aims of this thesis

The general aim of this thesis was to investigate and understand if and how Leukemia inhibitor factor (LIF) exerts HIV-1 suppressing activity.

The specific objectives were;

To investigate if LIF and its corresponding receptors gp130 and LIFR-α had potential systemic immunoregulatory effects during the course of HIV-1 infection in lymphoid tissue (paper I).

To investigate whether primary HIV-1 infected (PHI) individuals with sustained virological control post-cessation of ART had a higher initial LIF response during PHI as compared to those individuals who did not achieve a similar control of HIV-1 replication (paper II).

To investigate the pattern of immunoactivation by studying secretion of cytokines and chemokines during PHI and in the chronic phase of HIV-1 infection, after antiretroviral therapy termination (paper III).

To investigate the potential involvement of Stat proteins in the LIF induced anti-HIV-1 effect (paper IV).

Material and methods

For details about the material and methods used in this thesis, see respective paper.

cMAGI cells and MAGI assay

cMAGI (multinuclear activation of galactosidase indicator) cells are CXCR4 expressing HeLa cells transfected with CD4, CCR5 and HIV-LTR-β-galactosidase and function as a reporter system for HIV-1 replication (Chackerian B, *J Virol.* 1997;71:3932-3939). β-galactosidase is transcribed when the MAGI cells are infected with HIV. Addition of X-galactosidase, which reacts with β-galactosidase, subsequently results in a bluish staining of infected cells. The MAGI assay was performed as outlined in paper IV.

Ethical clearance

The studies included in thesis were performed 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 & Discussion

Background - potential role for LIF in HIV regulation in vivo

The focus of my research has been to investigate and understand if and how LIF exerts HIV-1 suppressing activity. When I started this project, Patterson *et al*, had just presented *in vivo* and *in vitro* work that showed that the expression of placenta-derived LIF was associated with protection from transmission of HIV-1 from mother to child. Furthermore, they showed that LIF inhibited HIV-1 replication *in vitro* in PBMC as well as in placenta and thymus explant models. We then though that it would be interesting to investigate if LIF could also play a cell-protective role in individuals already infected with HIV-1, and if LIF could be detected in other tissues than the placenta. We began with the lymphoid tissue, since that is where the majority of HIV-1 replication occurs and where most of the mediators in the immune system can meet and interact with each other. We also aimed to analyse plasma samples from HIV-1 infected indivduals since that may reflect LIF regulation at the systemic level.

Enhanced expression of LIF and its receptors, gp130 and LIFR-α, in primary HIV-1 infection.

In paper I, we investigate whether LIF and its receptors, gp130 and LIFR-α, had immunoregulatory effects during the course of HIV-1 infection, by looking at LT biopsies (tonsils and lymph nodes) from patients with PHI, chronic HIV-1 infection (cHI), long-term non-progressors (LTNP) and HIV-1 seronegative controls. LIF, gp130, LIFR-α and HIV-1 replicating cells were identified at the single cell level by immunohistochemistry and quantified by computerised *in situ* imaging in infected tissue and in healthy controls.

The frequency of LIF, gp130 and LIFR-α expressing cells in the extra follicular area of LT were significantly increased in PHI when compared to HIV-1 seronegative controls (Figure 7). Furthermore both the expression of gp130 and LIFR-α, but not LIF, were significantly increased in cHI as compared to HIV-1 seronegative controls. However, the LTNP-group showed a significantly decreased expression of LIF, gp130 and LIFR-α, as compared to HIV-1 seronegative controls. In addition, real-time RT-PCR data showed that LIF mRNA levels were elevated in LT sections from the PHI group while cHI, LTNP

and HIV-1 seronegative controls showed comparable amounts of LIF mRNA (Figure 7f).

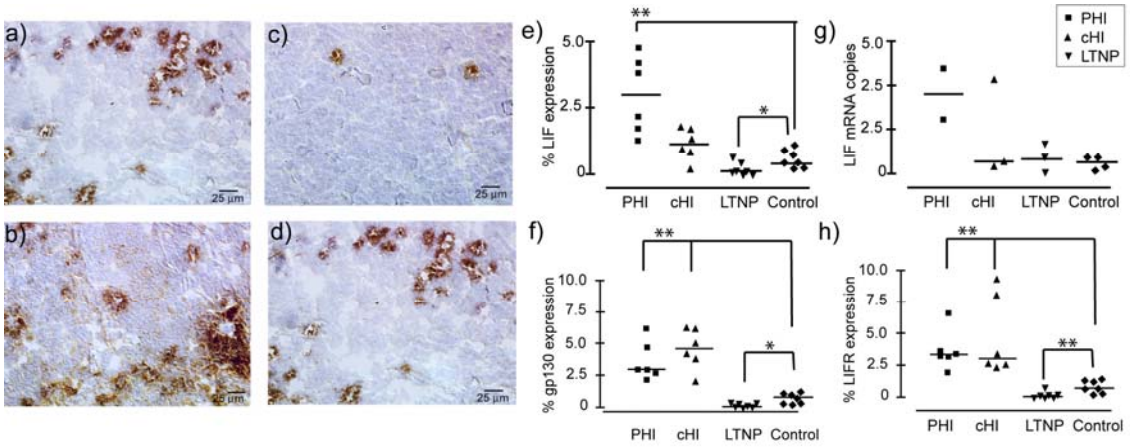

Figure 7. *Immunohistochemically stained sections from LT biopsies illustrating LIF expressing cell (stained brown) in* ***a)*** *and* ***c)*** *and gp130 (stained brown) in* ***b)*** *an* ***d)****. a) Note the high numbers of cells expressing LIF and gp130 in primary HIV-1 infection* ***a)*** *and* ***b)*** *as compared to HIV-1 seronegative control tissue* ***c)*** *and* ***d)****. Cells were counterstained blue with hematoxylin.* ***e)*** *LIF expressing cells in LT at different stages of HIV-1 infection and in HIV-1 seronegative controls assessed by in situ imaging presented as median values of the percentage of stained area out of the total cellular area.* ***f)*** *LIF mRNA copies/200 000 GAPDH copies in LT cellular extract from different stages of HIV-1 infection and in HIV-1 seronegative controls as assessed by RT-PCR. Incidences of lymphoid cells expressing* ***g)*** *gp130 and* ***h)*** *LIFR-α in LT as assessed by in situ imaging, and expressed as median values of the percentage of stained area out of the total cellular area. Statistical differences between the differerent groups were assessed by a two-tailed Mann-Whitney test and are indicated by * ($p<0.05$) and ** ($p>0.01$), respectively. ■primary HIV-1 infection (PHI), ▲chronic HIV-1 infection (cHI), ▼long-term non-progressors (LTNP) and ♦HIV-1 seronegative controls.*

In paper II we assessed the concentration of LIF and of the soluble form of gp130 (sgp130) in consecutively obtained HIV-plasma samples collected during the PHI phase and after cessation of ART from HIV-1 infected individuals that were included in the QUEST study, the first placebo controlled treatment trial in acutely HIV-1 infected[258]. The QUEST cohort is very unique since the patients were enrolled very early after exoposure to HIV-1, the first sample was taken approximately 11 days from onset of PHI symptoms. We selected a group of *"controllers"* defined as subjects with a viral set point of <1000 HIV-1-RNA copies/ml plasma 6 months post-stopping ART (> 1 year after the onset of infection) and a group of *"non-controllers"* who were defined as those with a corresponding viral set point of >9000 copies of HIV-1-RNA copies/ml plasma 6 months post-stopping ART (>1 year after onset of infection). There was a significant difference in viral load between the two groups already at onset of the study. We investigated whether the *"controllers"* showed higher initial LIF levels

when compared to *"non-controllers"*. Plasma from acutely symptomatic EBV-infected subjects and from a group of HIV-1-seronegative healthy individuals served as controls.

LIF was significantly increased during the PHI phase (day 1 to week 28) in HIV-1 infected individuals when compared to HIV-1-seronegative healthy controls (Figure 8a). However, there was no significant difference in the LIF plasma concentration between *"controllers"* and *"non-controllers"*, although the median value showed a trend towards a higher LIF concentration in *"non-controllers"* when compared to *"controllers"* from day minus 7 to day 1 post initiation of ART. Overall the kinetic response pattern was comparable between the two groups.

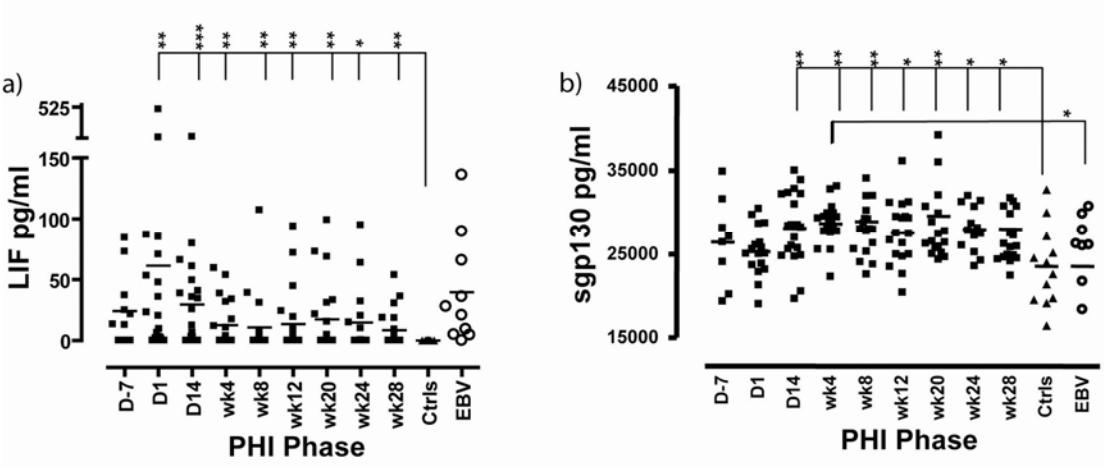

Figure 8. *Distribution and mean* ***a)*** *LIF plasma levels and* ***b)*** *sgp130 levels during PHI. Differences in levels between HIV-1 infected subjects and uninfected healthy controls or HIV-1 infected subjects and acutely EBV infected individuals were assessed by Wilcoxon Rank test with 95% CI, statistically significant differences are indicated by * ($p<0.05$), ** ($p>0.01$) and *** ($p<0.001$), respectively.*

Soluble gp130 was significantly increased in HIV-1 infected individuals between day 14 and week 28, during the PHI phase, as compared to HIV-1-seronegative healthy controls (Figure 8b). No significant difference between *"controllers"* and *"non-controllers"* was found. Furthermore, the median levels of sgp130 increased between day one and day 14 during the PHI phase while the detectable levels of LIF were decreasing during the same time period.

Together these two studies (paper I and II) showed that induction of LIF occurred early in the PHI phase and that it was a part of an early virally induced pro-inflammatory response rather than an adaptive immune response against HIV-1. In fact, LIF protein expression was upregulated in one patient as early as 2 days after onset of anti-retroviral syndrome (paper I) and the peak of LIF in the plasma occurred already at day 7 before initiation of ART to day 1 post initiation

of ART (paper II). Plasma levels of both LIF and of sgp130 were significantly reduced during the ART cessation phase (Figure 8) which implicated that the viral reactivation occurring after treatment interruption did not induce the same magnitude of pro-inflammatory response as in PHI. This proposes a role for LIF in the innate immune response against HIV-1. Furthermore, the induction of LIF did not seem to be HIV-1 specific, since there were no significant differences in the LIF plasma concentration between acute EBV and PHI patients at day minus 7 to day 14 post initiation of ART (Figure 8).

The expression of gp130 and LIFR-α in LT correlated with the expression of LIF at various stages of HIV-1 infection with the exception of the chronic phase (paper I). This may be due to the persistent pro-inflammatory milieu present in LT throughout the course of HIV-1 infection[259] which may explain the upregulation of LIF receptors in cHI in contrast to LTNP which is associated with reduced inflammation [260]. Furthermore, it is not only LIF which utilizes gp130 and LIFR-α; all members of the IL-6 cytokine family [IL-6, IL-11, oncostatin M (OSM), ciliary neurotropic factor (CNTF) and cardiotrophin (CT-1] signal via the gp130 receptor. OSM, CNTF, and CT-1 causes hetereodimerisation of gp130/LIFR-α and a third cytokine specific receptor subunit which results in signal transduction. LIF binds to LIFR-α and this complex dimerize with gp130 which generates a high affinity complex needed to induce cell signalling[160].'

The median detectable levels of plasma LIF were decreasing between day one and day 14 during the PHI phase while the median levels of sgp130 increased at day 14 (Paper II). One may therefore speculate that sgp130 is released into the circulation to block and neutralize systemically released LIF, in order to regulate the biological activity of LIF[261, 262]

Positive correlation between viral load and LIF expression in lymphoid tissue.

A positive significant correlation between levels of plasma HIV-1 viral load and the expression of LIF (Figure 9a) in LT (paper I) as well as a positive significant correlation between levels of plasma HIV-1 viral load and plasma LIF concentration (Figure 9b) were found (paper II). This suggests that it is the viral dissemination, which drives the LIF production.

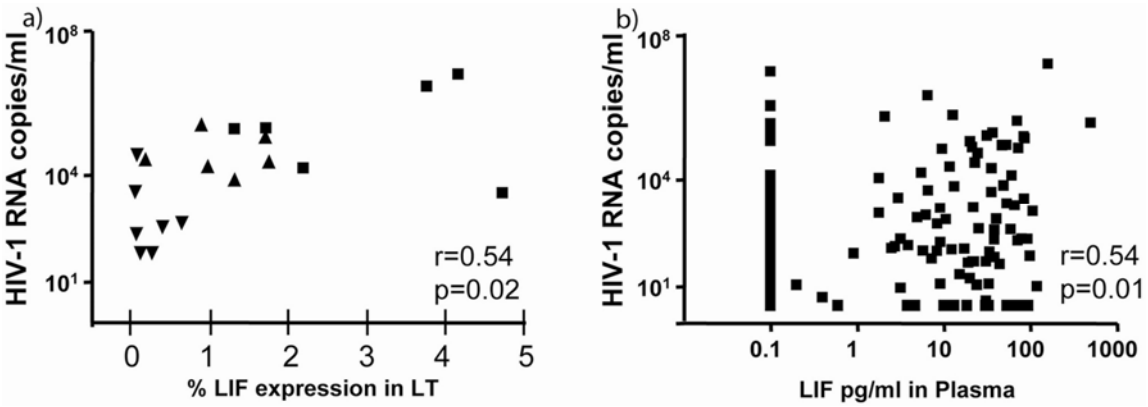

Figure 9. *Positive correlation between viral load and LIF. Plasma viral load is plotted as a function of* ***a)*** *LIF protein expression in LT from HIV-I infected individuals and* ***b)*** *plasma levels of LIF from HIV-I infected individuals.*

HIV core antigen expression was limited to $CD4^+gp130^-$ cells in lymphoid tissue.

Multiple fluorescent staining, in LT from PHI patient, was used to identify the phenotype of HIV-1 replicating cells (paper I). Confocal microscopy revealed that HIV-1 core antigen (p24) was evident mainly in $CD4^+$ cells but there was also a few percent that were $CD4^-$. However, significantly fewer cells expressing gp130 (both $CD4^+$ and $CD4^-$) expressed HIV-1 core antigen. Despite the fact that more than 50% of the total $CD4^+$ cells expressed gp130, less than 5% of the total HIV replicating cells in LT were $gp130^+$ (Figure 10). Furthermore, we could not demonstrate signs of HIV-1 replication in LIF expressing cells. This implicates that LIF mediates a certain control of HIV-1 replication via gp130 and LIF may limit the ability of HIV-1 to replicate in $gp130^+$ cells that have received LIF signalling.

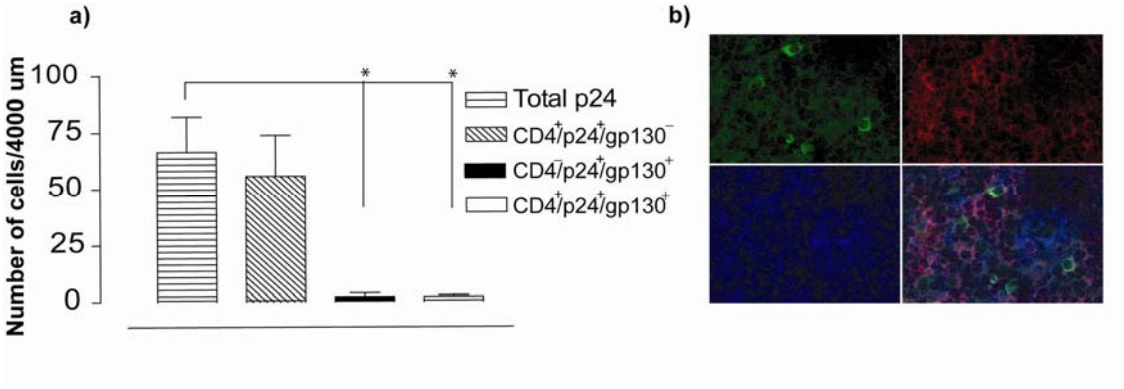

Figure 10. a) *Incidence of HIV-1 replicating cells (p24)/4000 μm^2 LT. Note that both $p24^+/CD4^-/gp130^+$ cells and $p24^+/CD4^+/gp130^+$ cells express significantly less HIV-1 core antigen as compared to $p24^+/CD4^+/gp130^-$ cells ($p<0.05$).* ***b)*** *Confocal images of LT sections from a PHI patient illustrating staining of HIV-1 core antigen (green, top left panel), CD4 (red, top right panel) and gp130 (blue, lower left panel). Overlay of all three stainings demonstrating that none of the HIV-1 core antigen expressing cells co-stained for gp130 (lower right panel).*$p<0.05$*

Phenotypic characterization of cells expressing LIF, gp130 and LIFR-α.

Two-colour staining in LT showed that LIF, gp130 and LIFR-α were present on $CD3^+$, $CD4^+$ and $CD8^+$ cells (paper I). LIF was expressed in approximately 10 to 50 % of all $CD3^+$, $CD4^+$ or $CD8^+$ cells whereas gp130 or LIFR-α was expressed on approximately 10 to 60 % of all $CD3^+$, $CD4^+$ or $CD8^+$ cells in LT. To further characterize the phenotype of cells expressing gp130 and LIFR-α, PMBCs, separated from healthy blood donors were stained for different cell-surface markers. Flow cytometry analyses revealed that gp130 was expressed on approximately 65 % of all $CD3^+CD4^+$ T-cells (47-80%), 63% of all $CD3^+CD8^+$ T-cells (50-76%) and on almost all $CD4^+CD14^+$ monocytes (70-100%). Furthermore, gp130 was expressed on very few if any $CD3^-CD19^+$ B-cells and $CD3^-CD56^+$-NK-cells. This is in agreement with data from Oberg *et al* showing similar frequency of gp130 expression in blood cells[263]. LIFR-α was expressed to a much lower extent then gp130; approximately 5% of $CD3^+CD4^+$T-cells, 35% of $CD3^+CD8^+$ T-cells and 65% of $CD4^+CD14^+$ monocytes. This suggests that, in blood, it is mainly monocytes that are sensitive to the anti-HIV-1 effect of LIF.

Since our data showed that HIV-1 core antigen (p24) was significantly less expressed in $CD4^+/gp130^+$ cells as compared to $CD4^+/gp130^-$ cells and since $CD4^+$ T cells are among the main HIV-1 target cells *in vivo*, we went on to further characterize $CD3^+CD4^+gp130^+$ cells in blood (Figure 11). It was however, not possible to further characterize $CD4^+CD14^+$ cells because the expression of gp130 was down-regulated on these cells when they were put in culture.

The $CD3^+CD4^+gp130^+$ cells did primarily express CXCR4, but a few percent expressed CCR5. They were mainly CD45 RA than CD45RO positive and did not express CD25, CD69 (activation markers). Nor did they express CD57 (a marker for replicative senescence). They expressed CCR7 and CD62L, which allow them to “home”/enter lymph nodes, and the co-stimulatory molecules CD27 and CD28. Thus, it was mainly naïve cells that expressed gp130 in blood. It would be interesting to find out if the expression of gp130 is altered in blood from HIV-1 infected individuals and if gp130 expressing cells are “protected“ from HIV-1 infection in blood as has been shown in LT. We are hoping to be able to answer these questions in the near future.

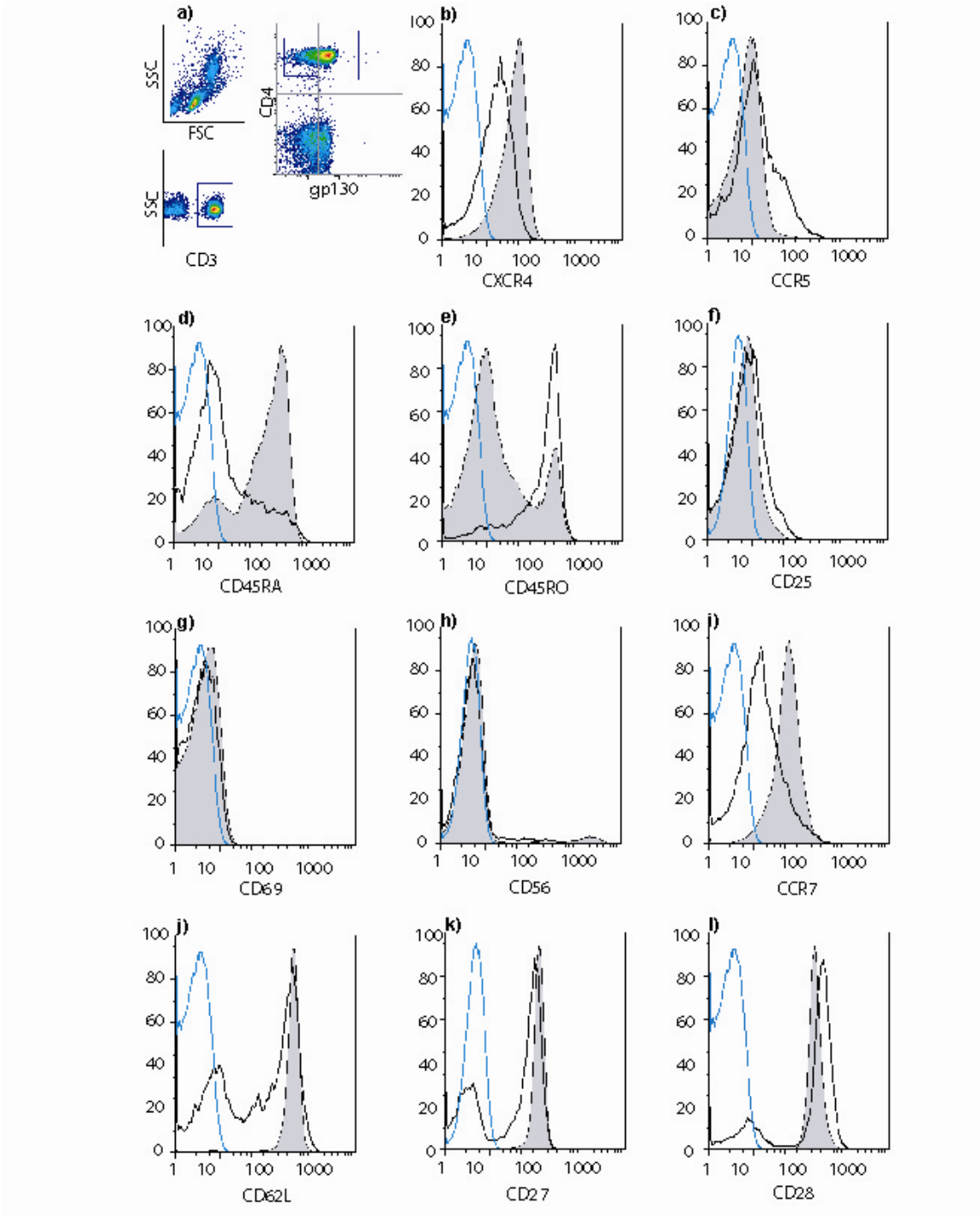

Figure 11. The graph shows facs analysis of PBMCs. **a)** A gate was sent on PBMCs based on size and granularity, thereafter a $CD3^+$ cell-gate was set followed by a $CD4^+gp130^+$ cell-gate (gray) and a $CD4^+gp130^-$ cell-gate (black line). The cells were further caracterized for **b)** CXCR4, **c)** CCR5, **d)** CD45RA, **e)** CD45RO, **f)** CD25, **g)** CD69, **h)** CD56, **i)** CCR7, **j)** CD62L, **k)** CD27 and **l)** CD28 expression. The blue line corresponds to unstained cells.

It is not the lack of proinflammatory cytokines and chemokines during PHI that is the problem in HIV-1 infection.

In paper III we further investigated the pattern of immunoactivation during PHI as well as after cessation of antiretroviral therapy, in the same cohort that was used in paper II. Cytokine and chemokine levels in plasma from the two different groups, *"controllers*" and *"non-controllers"* (termed responders and non-responders, respectively in the paper) were analyzed by Luminex. *"Non-controllers"* had significantly higher levels of IFN-γ, TNF-α, IL-1β, IL-10 and Eotaxin, respectively, than *"controllers"* during the PHI phase. Furthermore, there was a positive correlation between HIV-1 RNA and IFN-α, TNF-α, IL-1β, MIP-1α and MIP-1β, respectively. On the contrary, "controllers" had significantly higher levels of IFN-γ, MIP-1β and MCP-1 than *"non-controllers"* after cessation of ART (Figure 12). This suggests that the problem during PHI is not lack of a certain immune mediator as seen in many other infections. For example, during the acute phase of measles infection there is lack of IL-12[264, 265] whereas EBV infection produces virally engineered IL-10 to trick the immune system[266]. Thus for HIV-1 infection it looks like there are too much and too many mediators. However, those individuals that do control the infection seem to have a recall

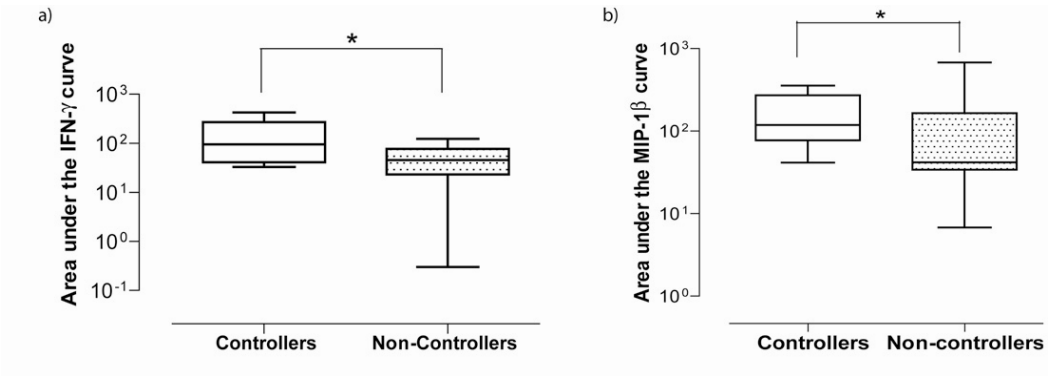

Figure 12. The graph shows the area under the **a)** IFN-γ curve and **b)** MIP-1β curve during the post-stopping antiretroviral treatment phase. Concentration of IFN-γ and MIP-1β were significantly higher in "controller" as compared to "non-controller" *p<0.05.

response to the virus, since they produce IFN-γ and chemokines. This can be compared to *in vitro* studies where PMBCs from HIV-1 infected individuals produce multiple cytokines and chemokines at the single cell level after restimulation *in vitro* with HIV-1 peptide pools. Therefore a polyfunctional $CD4^+$ and $CD8^+$ induced HIV specific response is associated with better control of

HIV-1 infection as compared to $CD4^+$ and $CD8^+$ T cells producing one single cytokine or chemokine[199]. Furthermore, the viral events during PHI seemed to determine the viral set-point determined after more than 2 years, despite an immediate initiation of ART. Thus initial virus-immune system interaction seems to regulate the long term viral replication level and thereby the disease related prognosis.

LIF inhibited HIV-1 infection and decreased uptake of HIV-1 antigen in cMAGI cells.

In paper IV we went on to study the mechanism of the LIF anti-HIV-1 activity seen in earlier studies. We used CXCR4- and gp130-expressing cMAGI cells transfected with CD4, CCR5 and HIV-LTR-β-galactosidase as a model system to investigate the potential involvement of Stat proteins in the anti-HIV-1 effect of LIF. The advantage of using these cells was that they did not require activation prior to HIV-1 infection and that it was a robust, reproducible model system that could be used to measure HIV-1 infectivity[267-269]. This was in contrast to primary naïve $CD4^+$ T cells which required activation in order to become susceptible to HIV-1 infection *in vitro.* Activation of $CD4^+$ T cells with anti-CD3, anti-CD28 stimulation resulted in down regulation of gp130. Furthermore, anti-CD3, anti-CD28 stimulation as well as PHA stimulation resulted in Stat phosporylation[270].

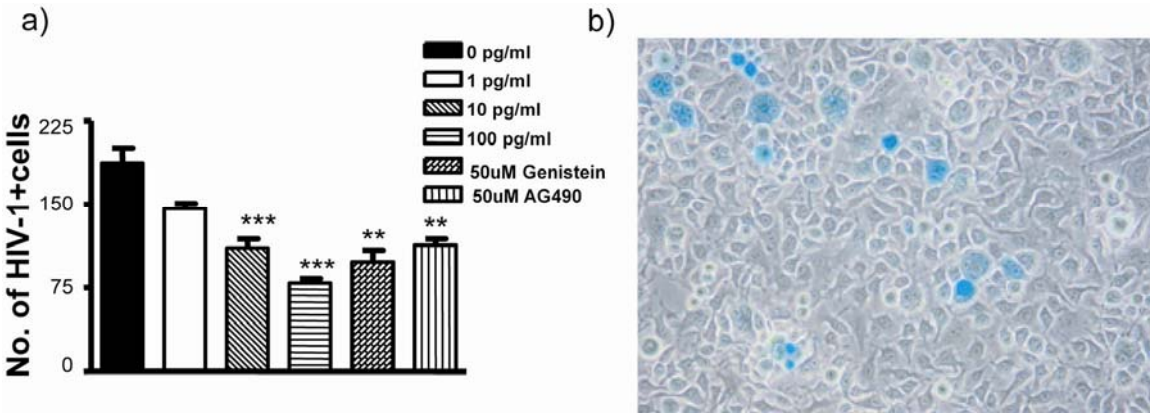

Figure 13. a) *Dose dependent inhibition of HIV-1 in LIF treated cMAGI cells. Treatment of cMAGI cells with Genistein (a protein kinase inhibitor) or AG490 (a Jak inhibitor) prior to exposure of HIV-1 resulted in significant inhibition of HIV-1 infection. ** p<0.01 and *** p<0.001.* ***b)*** *The picture shows HIV-1 infected cMAGI cells (blue). β-galactosidase is transcribed when the cMAGI cells are infected with HIV-1 and addition of X-galactosidase results in a bluish staining of infected cells when X-galactosidase reacts with β-galactosidase.*

Treatment of cMAGI cells with LIF prior to HIV-1 exposure resulted in significantly lower numbers of HIV-1 infected cells as compared to non-treated

cells (Figure 13) In addition, fluorescent staining of LIF treated and/or HIV-1 exposed cMAGI cells showed that treatment with LIF prior to HIV-1 exposure resulted in a statistically significant decrease in the number of HIV-1 p24 expressing cells that indicated a LIF mediated diminished uptake of HIV-1 particles. This observed phenomenon was not due to alteration in expression of the main HIV-1 receptors (CD4, CCR5 and CXCR4) nor to any major changes in the expression levels of the LIF receptors, (gp130 and LIFR-α) as determined by flow cytometry. In addition, cells treated with LIF did not induce or suppress production of IL-1β, IL-6, TNF-α, RANTES, MIP-1α, MIP1-β or SDF-1, as determined by assessment of cell culture supernatatnts by Luminex or ELISA. One can therefore conclude that the anti-HIV-1 effects detected in this study were not due to release of the above mentioned cytokines or chemokines which are known to have suppressive or inducing effects on HIV-1 replication [20, 161, 271, 272]. A transduction assay with murine amphotropic retroviral particles containing the MSCV-IRES-GPF expression vector was used to study whether the ability of LIF to affect the uptake of HIV-1 particles could be generalized to other retroviruses. However there were no differences in uptake between LIF treated and untreated cells, indicating that LIF did not have a general retroviral activity, but might have had a selective anti-retroviral activity since LIF affected the uptake of HIV-1 (paper IV) and it also affected the uptake of feline immunodeficiency virus (Patterson unpublished data).

LIF and HIV-1 induced phosphorylation of Stat 3 in cMAGI cells.

Western blot analysis performed on lysed cMAGI cells exposed to HIV-1 and/or treated with LIF revealed that Stat 3 (Figure 14) but not Stat 1 or Stat 5 was phosphorylated. Furthermore, if the cMAGI cells were treated with LIF prior to HIV-1 exposure, the HIV-1 mediated Stat 3 phosphorylation was down regulated (Figure 12). We used the Jak inhibitory agent, AG490 and the tyrosine kinase inhibitor, Genistein, to confirm the effect of LIF on the Jak/Stat signaling pathway. Reduced phosphorylation level of Stat 3 was evident in both AG490 and Genistein treated cells as compared to untreated cells. As for now, we do not know the exact mechanism behind this interference with the Jak/Stat pathway. However, one can speculate that there are downstream effects of the LIF-mediated signalling that block the HIV-1 mediated signalling. LIF induced

Stat 3 phosphorylation might compete for co-activators needed to complete the HIV-1 replication cycle.

The HIV-1 regulatory protein Tat is very important to enhance gene expression in the infected host cells. Tat binds directly to the TAR region of the LTR and also associates with other cellular co-factors like CyclinT1, SP1, CREB-binding proteins (CBP)/p300 and pCAF. Furthermore, CBP/p300 and pCAF have been shown to associate with both Stat proteins and with Tat[41]. Stat 1 binds to CBP/p300 both with its N- and C-terminus ends. The C- terminus end contains the transactivation domain which also binds to the Adenovirus oncoprotein E1A. IFN-γ decreases early adenovirus mRNA levels, which are dependent upon the adenovirus E1A protein. The antiviral effect of IFN-γ is thought to be a consequence of competition for binding to CBP between activated Stat 1 and E1A[273, 274]. LIF induced Stat 3 phosphorylation might compete for co-activators such as CBP/p300 in a similar way as demonstrated in the Adenovirus system. LIF thus affected HIV-1 infection rate via reduced Stat 3 phosphorylation that might in turn lead to lack of important co-activators needed to complete the HIV-1 replication cycle.

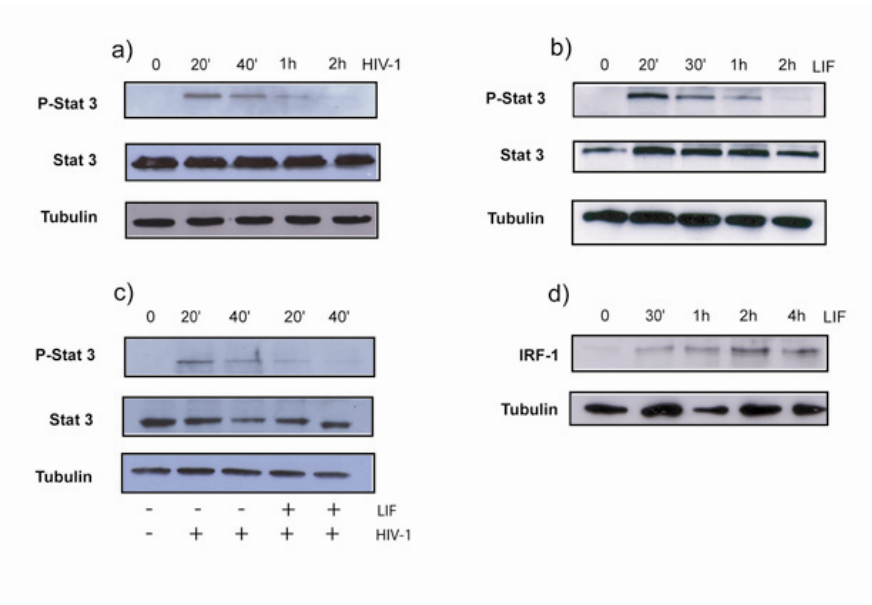

Figure 14. *Western blot performed on lysed cMAGI cells revealed that both* ***a)*** *HIV-1 and* ***b)*** *LIF phosphorylated Stat 3 in a time dependent manner.* ***c)*** *Pre-treatment with rhLIF down regulated the HIV-1 mediated Stat 3 phosphorylation after both 20 and 40 min of HIV-1_{BaL} exposure.* ***d)*** *LIF activated IRF-1 expression in a time dependent manner, ranging from 30 min to 4 h, with a peak at 2 h of LIF stimulation. Tubulin and unphosphorylated Stat 3 was used as loading control.*

In this study we also showed that cMAGI cells treated with LIF induced expression of the transcription factor IRF-1 (Figure 14). The anti-HIV-1 effects

seen by CAF and IFN-γ have been proposed to partly be mediated by enhanced expression of IRF-1[128, 275]. Expression of IRF-1 can be greatly enhanced by virus infection and by several cytokines[276-278]. Within the 5' region of the HIV-1-LTR-region there is a binding site for IRF-1[275]. Furthermore, HIV-1 induces IRF-1 expression prior to Tat production and IRF-1 can activate transcription of Tat, which in turn amplifies LTR-directed gene expression. Marsili *et al* have suggested that HIV-1 may have evolved a strategy to turn on the activation of IRF-1 before massive IFN-and IFN-stimulated gene activation occur thereby counteracting the host immune defence[279]. One may speculate that the kinetic response of IRF-1 induction may also be crucial for LIF mediated anti-HIV-1 effect. If LIF induces IRF-1 expression prior to HIV-1 infection, this might in turn alter HIV-LTR transcription. However, if HIV-1 exposure induces IRF-1 expression prior to LIF induction, HIV-1 may counteract the antiviral effect of LIF. This goes hand in hand with the observations that LIF has to be present prior to HIV-1 in order to have a suppressive effect on HIV-1 replication.

Stat 3 siRNA reduced p24 production in PBMC.

We next investigated if HIV-1 replication was affected by blocking the Stat 3 signaling pathway in primary cells. PBMCs were transfected with siRNA oligonucleotides directed against Stat 3 or scrambled siRNA prior to HIV-1 infection. Silencing of Stat 3 resulted in significantly less viral replication as determined by p24 production in cell culture supernatants (Figure 15). In order to calculate the relative changes in Stat 3 mRNA with siRNA treatment, we determined the threshold cycle numbers (Ct) where the fluorescence intensity of the PCR probes exceeded background levels (Figure 14, horizontal broken line) and by using the $2^{-\Delta\Delta Ct}$ equation, previously described [280], we showed a 34-fold and a 55-fold drop of Stat3 mRNA in cells transfected with Stat 3 siRNA prior to HIV-1 infection as compared to non-transfected but HIV-1 infected cells. Together, this shows that the Jak/Stat signaling pathway is important for the HIV-1 replication cycle and that LIF interferes with it.

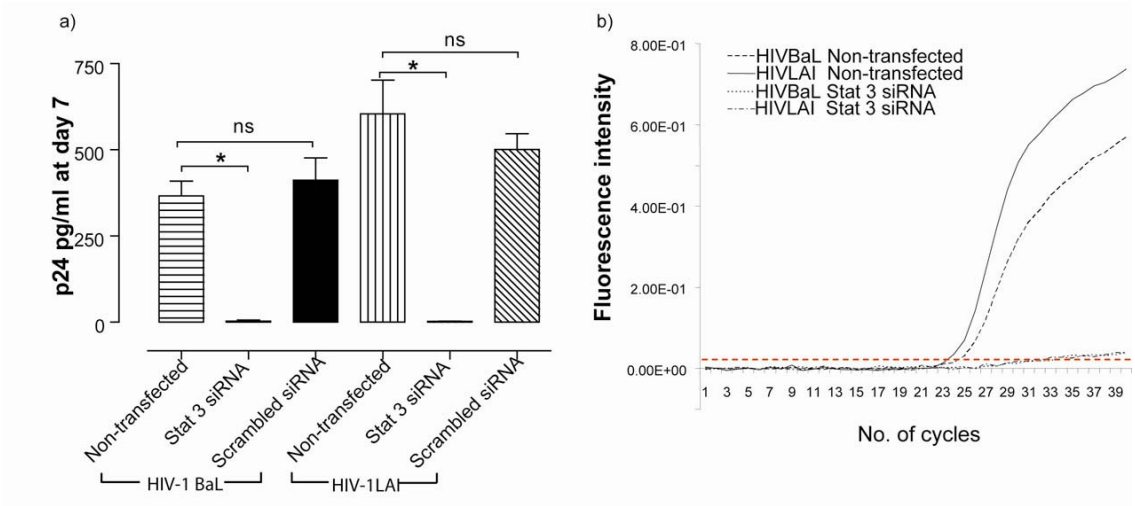

Figure 15. a) *The graph illustrates that PBMCs transfected with Stat 3 siRNA for 6 h prior to 1 h exposure to HIV-1 $_{BaL}$ or HIV-1$_{LAI}$ expressed significantly less HIV p24 antigen, on day 7, as compared to non-transfected HIV-1 infected cells. There were no significant differences in p24 expression between non-transfected and scrambled transfected cells. *p<0.05* ***b)*** *Confirmation of Stat 3 mRNA silencing was determined using quantitative real-time RT-PCR for Stat 3 mRNA. The threshold cycle numbers (Ct) were determined,and by using the previously described equation $2^{-\Delta\Delta}$ we showed a 34-fold and a 55-fold drop of Stat3 mRNA in cells transfected with Stat 3 siRNA for 6 hours prior to HIV-1$_{BaL}$ (broken line----) or HIV-1$_{LAI}$ (solid line ——) infection, respectively, as compared to non-transfected but HIV$_{BaL}$ (dash and dotted line -·-·-) or HIV-1$_{LAI}$ (dotted line·····) infected cells.*

Concluding remarks

1) High expression of LIF, gp130 and LIFR-α was observed in lymphoid tissue (LT) biopsies from individuals with PHI. A positive correlation between plasma viral load and LIF expression in LT was found. Even though more than 50% of the total $CD4^+$ cells in lymphoid tissue expressed gp130, less than 5% of the total HIV replicating cells ($p24^+$) in lymphoid tissue were $gp130^+$. Significantly less HIV-1 viral replication (identified by HIV core antigen/p24 expression) was observed in $CD4^+$ cells expressing gp130 in LT as compared to $CD4^+gp130^-$ cells.

- LIF is expressed early in the acute phase of HIV but not in the chronic phase of HIV, nor in lymphoid biopsies from LTNP. The fact that LIF expressing cells could be demonstrated in LT already 2 days after onset of acute retroviral syndrome suggets that LIF is a mediator of the early immune response against HIV-1. HIV-1 replication was less pronounced in cells expressing the LIF signalling receptor, gp130, indicating that LIF could mediate a certain control of HIV-1 infection in $CD4^+gp130^+$ cells in LT

2) Increased levels of LIF and sgp130 were found in plasma samples from HIV-1 infected patients during the PHI phase. No significant differences in the LIF plasma concentrations between *"controllers"* (<1000 HIV-1-RNA copies/ml plasma 6 months post-stopping ART) and *"non-controllers"* (>9000 HIV-1-RNA copies/ml plasma 6 months post-stopping ART) were found. The median value showed a trend towards a higher LIF concentration in *"non-controllers"* when compared to *"controllers"* in the acute phase of HIV-1 infection from day minus 7 to day 1 post initiation of ART. However, *"non-controllers"* had higher levels of viremia when compared to *"controllers"* during the same time period. Furthermore a positive correlation between plasma viral load and LIF plasma levels from samples taken during the PHI phase was found.

- High levels of LIF were thus seen at the initial stages of PHI associated viral dissemination. This suggests that LIF may be a part of the early

virally induced immune response. However, LIF plasma levels during PHI did not predict low levels of HIV-1 viremia after discontinuation of ART (> 1 year after the onset of infection). Furthermore, LIF was not increased following ART interruption.

3) A positive correlation between plasma levels of HIV-1 RNA and IFN-α, TNF-α, IL-1β, MIP-1α and MIP-1β was found in plasma from HIV-1 infected individuals who were in the primary phase of infection. "*Non-controllers*" had significantly higher plasma concentration of HIV-1 RNA, IFN-γ, TNF-α, IL-1β, IL-10 and Eotaxin, respectively, than the *"controllers"* during PHI. After cessation of ART, the levels of cytokines and chemokines were reduced as compared to the levels seen during the PHI phase. HIV-1 infected individuals that controlled their infection after cessation of ART had higher plasma levels of IFN-γ and MIP-1β as compared to individuals that did not control their HIV-1 infection.

- A high level of immune activation during PHI is associated with high HIV-1 RNA levels rather than a better control of the viral replication. In addition, patients with better control of the virus after cessation of ART might have a recall response to the virus, since they produce IFN-γ and MIP-1β, which are suggested to be beneficial for the host to control the HIV-1 replication[93]. The viral interaction with initial immune response during PHI seemed to determine the viral set-point after more than 2 years, despite an immediate initiation of ART.

4) Treatment of cMAGI cells with LIF prior to HIV-1 infection resulted in a dose dependent reduction in numbers of HIV-1 infected cells when compared to untreated cells. Additionally, Jak/Stat inhibitors as well as siRNA knockdown of Stat 3 reduced the numbers of HIV-1 infected cells and reduced the production of HIV-1 core antigen/p24 when compared to untreated cells, respectively. Furthermore, both LIF and HIV-1 induced phosphorylation of Stat 3 while LIF pre-treatment resulted in a down modulation of the HIV-1 mediated Stat activation. LIF also induced expression of IRF-1, a transcription factor known to be important for HIV-1 replication and involved in the anti-HIV-1 effects seen by IFN-γ and CAF[128, 275, 279].

- LIF had suppressive effects against HIV-1 infection if present prior to infection and we propose that this effect was mediated by interferance with the HIV-1 mediated Jak/Stat activation. The Jak/Stat signalling pathway is crucial for the HIV-1 replication cycle and many studies have shown the involvement of the Jak/Stat signalling pathway during the course of HIV-1 infection[138, 184, 281-285].

In conclusion, LIF is one of the players of the innate immune response during HIV-1 infection and is, like many other cytokines, bifunctional. LIF showed HIV-1 suppressive action, if present prior to HIV-1 infection, and enhanced viral activity, if present after established HIV-1 infection. This is in agreement with data presented by both Broor *et al* and Patterson *et al*[256, 257]. LIF is not a potential systemic immunoregulator but may act locally to prevent HIV-1 infection if present prior to HIV-1 infection. Moreover LIF has gone through Phase I/II trials where it has been shown to be non toxic even in very high concentrations[251]. Therefore, LIF has a potential to function as a microbicide.
Since there are many steps within the life cycle of HIV-1 that are dependent upon phosphorylation and since the Jak/Stat signaling pathway has been proposed by us and others to play an important role during HIV-1 infection it would be very interesting to pin-point exactly where/how the Jak/Stat signaling pathway is crucial for the life cycle of HIV-1. Perhaps the results from such studies could gain information about new potential target sites for novel anti-HIV-1 drugs.

Populärvetenskaplig sammanfattning

I min avhandling har jag kartlagt Leukemia Inibitor Factors (LIF's) betydelse för prognos och sjukdomsprogress vid HIV-1 infektion.

LIF är ett cytokin, en slags signalsubstans som immunceller producerar i syfte att kommunicera med varandra. LIF upptäcktes av Dr Metcalf år 1987. Han och hans medarbetare visade att LIF kunde förhindra cancerceller att dela på sig genom att få dessa att mogna ut (differentiera). Idag vet man att LIF medverkar i många olika processer i kroppen. LIF reglerar tex tillväxt och differentiering av embryonala stamceller, blodceller, nervceller och fettceller. LIF är även en viktig molekyl i immunförsvaret, där den kan fungera antingen pro- eller anti-inflammatoriskt beroende på hur omgivningen ser ut.

När jag började med detta projekt hade Dr Bruce Patterson just visat att celler från moderkakan hos HIV-infekterade kvinnor som inte överförde viruset till sina barn uttryckte mer LIF än celler från de kvinnor som överförde viruset. Dessutom visade han att om cellerna behandlades med LIF i provrör innan de infekterades med HIV så förhindrade LIF virus-replikation. Vi ville därför undersöka om vi kunde detektera LIF även i andra vävnader, om LIF kunde förhindra virus-replikation i celler som redan var HIV-infekterade och vi ville dessutom försöka förstå hur LIF hämmar virus-replikation. Eftersom HIV främst replikerar i lymfoid vävnad valde vi att undersöka denna. Vi undersökte även plasma från HIV infekterade patienter under den akuta HIV fasen samt efter behandlingsavbrott, för att få svar på hur det ser ut systemiskt.

Uttrycket av LIF och dess receptorer (gp130 och LIFR-α) var förhöjt i lymfoid vävnad från individer i den akuta fasen av HIV jämfört med lymfoid vävnad från friska individer. De celler som vanligtvis blir infekterade av HIV uttrycker ytmolekylen CD4 som viruset använder för att ta sig in i målcellen. Vi såg att de $CD4^+$ celler som också uttryckte gp130 på sin yta inte i lika stor utsträckning blev infekterade av HIV som de $CD4^+$ celler som inte uttryckte gp130. Vi tror därför att LIF kan skydda celler, som uttrycker gp130, från HIV-infektion.

Koncentrationen av LIF och lösligt gp130 var också mycket högre i plasma från HIV-infekterade individer som var i den akuta HIV-fasen jämfört med friska individer och även med de nivåerna vi detekterade i plasma från kroniskt HIV-infekterade individer under behandlingsavbrott. Vi såg ingen skillnad i LIF-nivåerna i plasma hos de individer som kontrollerade sin HIV infektion jämfört

med dem som inte kontrollerade sin infektion. Vi såg ett positivt förhållande mellan virusmängd och LIF-uttryck vilket troligen betyder att det är viruset som driver utsöndring av LIF.

Vi har också analyserat dessa plasmaprover för andra cytokiner och kemokiner (signalsubstanser som är viktiga i immunförsvaret) och såg att under den akuta fasen av HIV-infektion var nästan alla dessa signalsubstanser avsevärt förhöjda jämfört med nivåerna under behandlingsavbrotts-fasen. Mängden av de flesta av dessa signalsubstanser korrelerade även positivt med virus nivån. Problemet vid HIV-infektion verkar således inte vara att det saknas någon specifik cytokin eller kemokin. Snarare finns det för många substanser i för hög koncentration och denna miljö verkar gynna HIV. Emellertid såg vi att de individer som kontrollerade sin infektion efter behandlings-avbrottet producerade IFN-γ och MIP-1β, en cytokin respektive en kemokin som tidigare också har visats vara viktiga vid kontroll av HIV infektion.

Vi undersökte också hur LIF påverkar virus replikation. Vi såg att celler som förbehandlats med LIF före HIV-exponering blev infekterade i lägre grad jämfört med obehandlade celler. Vi undersökte också om den signaleringsväg som innefattar proteinerna Jak och Stat var viktig i detta sammanhang. Vi fann att både LIF och HIV aktiverar denna signalväg, samt att förbehandling av cellerna med LIF ledde till minskad HIV-aktivering av Jak/Stat signalvägen. Därnäst behandlade vi cellerna med preparat som blockerar denna signalväg och fann att de inte blev infekterade i samma grad som de obehandlade cellerna. Vi drog då slutsatsen att Jak/Stat är viktig för HIV-replikation och att LIF interfererar med HIVs förmåga att aktivera Jak/Stat signalvägen.

Sammantaget tyder dessa resultat på att LIF uttrycks/utsöndras i den tidiga fasen av HIV och därför kan vara en av många proteiner som tillsammans utgör det naturliga medfödda immunförsvaret. LIF verkar både ha en positiv och en negativ roll i immunförsvaret mot HIV. När HIV infektionen redan är etablerad har LIF förmodligen en negativ inverkan genom att aktivera replikation av HIV, men om LIF finns där innan HIV-infektion kan LIF förhindra upptag av HIV samt blockera virus-replikation via störning av HIVs förmåga att aktivera Jak/Stat signalvägen. Vi hoppas därför att LIF ska kunna fungera som en microbicid. Microbicider är ett samlingsnamn för produkter såsom geler, krämer och suppositorium som utvecklats för att förhindra lokal överföring av HIV och andra

sexuellt överförbara sjukdomar när de appliceras i vagina eller ändtarm. Det råder stort hopp om att microbicider ska bli tillgängliga för individer i områden där HIV-infektion är vanligt förekommande.

Det skulle dessutom vara intressant att undersöka exakt var och när under HIVs replikationscykel som Jak/Stat signalvägen är viktig. Detta skulle förhoppningsvis kunna leda till att man hittar nya angreppspunkter för HIV-behandling.

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"Keep on rocking in the free world"
Neil Young

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