REGIONS OF THE CD127 CYTOPLASMIC TAIL NECESSARY FOR HIV-1 TAT-BINDING.

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Abstract

Impaired cell mediated immunity is the clinical hallmark of HIV infection yet the manner in which CD8 T-cells are disabled is not yet fully understood. IL-7 signalling is essential for normal CD8 T-cell development and function. Our lab has previously shown decreased expression of the IL-7 receptor a-chain (CD127) on circulating CD8 T-cells in HIV+ patients is mediated by the HIV Tat protein which results in poor CD8 T-cell function. Soluble Tat protein is secreted by infected CD4 T-cells and taken up by neighbouring uninfected CD8 T-cells through endocytosis. Once in the cytoplasm, Tat translocates to the inner leaflet of the cell membrane where it binds directly to the cytoplasmic tail of CD127 inducing receptor aggregation, internalization, and degradation by the proteasome. By removing CD127 from the cell surface, the HIV Tat protein is able to reduce IL-7 signaling and impair CD8 T-cell proliferation and function.

To determine which domain(s) in the cytoplamic tail of CD127 are required for interaction with Tat, a series of plasmids encoding for CD127 deletion mutants were successfully created. These series of mutant CD127 coding sequences were transfected into a eukaryotic expression system, the Jurakt cell line, where CD127 mutants were successfully expressed. Before determine which region on CD127 is required for Tat binding, an optimized Ni-NTA column system was used to successfully isolate histidine-tagged HIV-1 Tat at a high yield and purity from *E. coli.* This HIV Tat protein was used to treat the lysates of the Jurakt cells transfected with the panel of CD127 mutants. CD127 was then immunoprecipitated, followed by Western analysis of the immune complexes to detect Tat protein. Tat was immunoprecipitated with all CD127 mutants suggests neither tyrosine 449, box 1, the acidic region, serine region nor C-tail are specifically required for Tat binding to CD127.

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List of Abbreviations

Amino Acid

Amino Acid	3-Letters	1-Letter
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Weights and Measures

M—Moles
g—grams
m—meters
L—Litres
λ —Wavelength
c—centi
m—milli
µ—micro
n—nano
p—pico

A

aa—Amino acid
ADA—Adenosine deaminase
AIDS—Acquired immunodeficiency syndrome
Amp— Ampicillin
AP—Antarctic phosphatase
APCs—Antigen presenting cells
APS—Ammonium persulfate
ART—Anti-retroviral therapy

B

βTrCP—Beta-transducin repeat-containing protein
Bad—Bcl-2-associated agonist of cell death
Bax—Bcl-2–associated X protein
BCA—Bicinchoninic acid
Bcl-2—B-cell CLL/lymphoma 2
Bcl-xl—B-cell lymphoma-extra large
BglII—*Bacillus globigii* restriction enzyme II
Bim—Bcl-2-like protein 1
BSA—Bovine serum albumin

C

C—Carboxyl
c-CBL—Cellular casitas B-lineage lymphoma
c-Myb—Cellular myeloblastosis
CA—HIV Capsid protein, p24
CAF—CD8 antiviral factor
Cat. No.—Catalogue number
CCPs—Clathrin-coated pits
CCR5—CC chemokine receptor 5
CD—Cluster of differentiation
CD132—Common gamma chain; interleukin receptor
Cdk—Cyclin dependent kinase
CIS—Cytokine-*inducible SH2 protein*
CNS— Central nervous system
CMV—Cytomegalovirus
CpG—Phopho-cytosine-guanidine islands
co-IP—Co-Immunoprecipitation
CRH1—Cytokine receptor homology class I
CTD—Carboxyl-Terminal Domain
CTL—Cytotoxic T Lymphocyte
Cul5—Cullin 5
CXCR4—CXC Chemokine Receptor 4

D

DC—Dentritic Cells
DC-SIGN—Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin
DCIR—Dendritic Cell Immunoreceptor
ddH_20—Double distilled H_20
de novo—"A new"
DpnI—*Diplococcus pneumoniae* restriction enzyme 1
DNA—deoxyribonucleic acid
dNTP—deoxy Nucleotide Tri-Phosphates

E
EC—Elite controllers
ECD—Extracellular Domain
ECL—Enhanced chemiluminescence
EcoRI—*E coli* RY13 restriction enzyme RI
EDTA—Ethylenediaminetetraacetic acid
eGFP—Enhanced green florescent protein
EGTA—Ethylene glycol tetraacetic acid
Env—HIV envelop polyprotein
ER— Endoplasmic Reticulum
ERAD—Endoplasmic Reticulum Associated Degradation
ERK—mitogen-activated protein kinase 3
et. al.—"And others"

F
FADD—Fas-Associated protein with Death Domain
Fas—TNF receptor superfamily, member 6
FasL—Fas ligand
FCS—Fetal calf serum

G
GABP—GA Binding Protein
Gag—Group-specific Antigen Protein
GALT—Gut-associated lymphoid tissue
GE—General Electric
Gfi-1—Growth factor independence-1
GFP—Green florescent protein
GLUT-1—Glucose transporter 1
Golgi—Golgi Apparatus
gp120—Glycoprotein: 120 KDa (HIV)
gp160—Glycoprotein: 160 KDa (HIV)
gp41—Glycoprotein: 41 KDa (HIV)
GRE—Glucocorticoid response element

H

HCl—Hydrochloric Acid
HCV—Hepatitis C virus
HDAC—Histone deacetylase complex
HindIII—*Haemophilus influenzae* Rd restriction enzyme III
HIV—Human immunodeficiency virus
HPLC—High pressure liquid chromatography
HRP—Horseradish peroxidase
HS—Heparan sulphate
Hsp—Heat shock protein
HSPG—Heparan Sulphate Proteoglycan
HSV-8—Herpes Simplex Virus 8

I

i.e.—"That is"
IFN—Interferon
IL—Interleukin
IL-7R—Interleukin-7 receptor
IL-7Ra—Interleukin-7 receptor alpha chain
In vivo—"In life"
In vitro—"in glass"
Int—HIV Integrase
IPTG—Isopropyl β-D-1-thiogalactopyranoside
IRS—Insulin receptor substrate

J

JAK—Janus Kinase
JAKi—Inhibitor of Jak activity

K

Kb—Kilobase
KCl— Potassium chloride
K_D—Disassociation constant
KDa—Kilodalton
KIDR—Kinase insert domain receptor
Kip27—Kinase inhibitor 27 KDa

L

LB—Luria Broth
LFA—Leukocyte function associated antigen
LRP—Low Density Lipoprotein Receptor-Related Protein
LTNPs—Long term non-progressor
LTR—Long terminal repeat

M

M-tropic—Macrophage tropic HIV
MA—HIV matrix protein
MACS—Magnet assisted cell sorting
MAP-2—Microtubule-associated protein 2
MAPK—Mitogen-activated protein kinase
Mcl—Myeloid cell leukemia
mDC—Myloid dendritic cells
MDDC—Monocyte-derived dendritic cells
Mdm-2—Murine double minute-2
MG132—Carbobenzoxy-Leu-Leu-leucinal
MHCI—Major histocompatibility complex I
MHCII—Major histocompatibility complex II
mRNA—Messenger RNA

N

N—Number of replicates
N-terminal—Amino-terminal
NaCl—Sodium chloride
NaH_2PO_4—Sodium dihydrogen phosphate
NC—Nucleocapsid protein
Nef—Negative regulatory factor
Neo—Neomycin
Ni-NTA—Nickel-nitriloacetic acid
NIAID—National Institute of Allergy and Infectious Diseases
NIH—National Institute of Health
NP-40-- Nonidet P-40

O

OD_{450}—Optical Density at 450 nm
OD_{600}—Optical Density at 600 nm
OHRI—Ottawa Hospital Research Institute

P

P/O—Promoter operator
p7—HIV nucleocapsid protein
p15—HIV ribonuclease H
p17—HIV matrix protein
p24—HIV capsid protein
p31—Integrase
p55—HIV Gag polyprotein
p51—HIV reverse transcriptase protein

$p56^{lck}$—Lymphocyte cell specific kinase, 56 KDa
p66—HIV reverse transcriptase protein with RNaseH subunit
p300/CBP—300 KDa protein/CREB-binding protein complex
PBMCs—Peripheral Blood Mononuclear Cells
PBS—Phosphate Buffered Saline
PCAF—p300/CBP-associated factor
Pck—Phosphoenolpyruvate carboxykinase
PCR—Polymerase Chain Reaction
PD-1—Programmed cell death 1
pDC—Plasmacoid Dendritic Cells
PE—Phycoerythrin
PE-PC7—Pphycoerythrin-phycoerythrin cyanin 7
PI3K—Phosphoinositol-3-Kinase
PIC—Pre-integration complex
Pol—Polymerase
Prod. No.—Product number
Prot— HIV protease
PRR—Pattern recognition receptor
pTEFb—Positive transcription elongation factor b
PU.1—Purine-rich box binding protein 1
PVDF—Polyvinylidine fluoride
pY449—Phosphorylated tyrosine at position of 449
Pyk2—Protein tyrosine kinase 2

R
Rev—HIV regulator of virion expression protein
RIPA—Radio immunoprecipitation assay
RLU—Relative light units
RNA—Ribonucleic acid
ROD—Subtype A virus that was isolated from a Cape Verdian in 1986
RON—Recepteur d'origine nantais
RPMI—Roswell Park Memorial Institute (Media)
RPMI-10—Roswell Park Memorial Institute (Media) with 10% fetal calf serum
RPMI-20—Roswell Park Memorial Institute (Media) with 20% fetal calf serum
RRE—Rev response element
RT—HIV reverse transcriptase
RT-RNasH—HIV reverse transcriptase with RNasH subunit
RUNX1—Runt-related transcription factor 1

S
SOC—Super optimal broth with catabolic repressor
SCID—Severe combine immunodeficiency
SDM—Site-directed mutagenesis

SDS—Sodium dodecyl sulfate
SH2—Src homology domain 2
Shc—Src homology 2 domain containing
SIV—Simian immunodeficiency virus
SOCS—Suppressor of cytokine signalling
Sp1—Specificity protein 1
SNP—Single nucleotide polymorphism
STAT—Signal transducer and activator of transcription

T
TAR—Tat response element
Tat—Transactivator of transcription
Tat-1—HIV-1 Tat
Tat-2—HIV-2 Tat
Tat-6xHis-- HIV-1 Tat with C-terminal polyhistidine tag
TBST—Tris-buffered saline with Tween-20
T_{CM}—Central memory T
TCR—T cell receptor
T_{EM}—Effector memory T
TEMED—Tetramethylethylenediamine
TGF-β--Tumour growth factor beta
Tip60—Tat interactive protein, 60kDa
TLR—Toll-like Receptor
TNF—Tumour Necrosis Factor
Treg—Regulatory T cell
tRNA—Transfer RNA

U
U—International Standard Unit
Ub—Ubiquitin

V
vDNA—Viral DNA
VEGF—Vascular endothelial growth factor
VLA-4—Very late antigen 4
Vpr—Viral protein R
Vpu—Viral protein U

W
WCL—Whole cell lysis
WHO—World Health Orgainzation

Y

y449—Tyrosine at position of 449

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1. Chapter 1: General Introduction

1.1 HIV-1

1.1.1 History of HIV

In the early 1980s, the first recognized cases of the acquired immune deficiency syndrome (AIDS) occurred among young adults afflicted with opportunistic infections that, until then, had only been seen in setting of profound immune deficiency. At this time, AIDS did not yet have its name, but it quickly became obvious that all of these young adults were suffering from a common syndrome. By 1983, human immunodeficiency virus (HIV) had been identified as the cause of AIDS (1-3). By the mid 1980's, it became clear that the virus had spread, largely unnoticed, throughout most of the world (4,5). Throughout the duration of the pandemic, more than 36 million people have already died from HIV (World Health Organization; WHO).

On a global level, the HIV pandemic remains one of the most serious infectious disease challenges facing public health. Currently, 35.8 million people are living with HIV, with 2.4-2.9 million new infections occurring in 2010 alone (WHO). HIV/AIDS remains an issue of concern for Canada, it was estimated that there were a cumulative total of 77,620 persons diagnosed with HIV in Canada by the end of 2011 (Public Health Agency of Canada; PHAC, 2012). The number of people living with HIV (including AIDS) continues to rise, from an estimated 64,000 in 2008 to 71,300 in 2011 (an 11.4% increase) and 3,175 newly diagnosed infections were reported in 2011 alone with an estimate 2,250 to 4,100 occurring annually (PHAC, 2012).

1.1.2 The Virion

HIV is a member of the lentivirus family of animal retroviruses. And like many lentiviruses, HIV is capable of long-term latent infection of cells and short-term cytopathic effects which cumulatively produce fatal diseases that include wasting syndromes and central nervous system (CNS) degeneration.

1.1.2.1 Structure and Genes

An infectious HIV particle consists of two identical positive sense RNA strands, 9.2kb in size, packaged within a core of viral proteins and surrounded by a phospholipid bilayer envelope derived from the host cell membrane that also includes virally encoded proteins. All the structural proteins and envelope glycoproteins seen in figure 1 are encoded by the *gag and env* sequences respectively (6,7). Finally *pol* sequences encode viral replication proteins: reverse transcriptase, integrase, and viral protease enzymes (7). In addition to these typical retrovirus genes, HIV-1 also includes six other regulatory genes, namely, the *tat, rev, vif, nef, vpr,* and *vpu* genes whose products regulate viral reproduction in various ways (6,7). See figure 2 for the HIV-1 genome organization.

1.1.2.2 Life cycle

The viral particles that initiate infection are usually in the blood, semen, or other body fluids of one individual and are introduced into another individual by sexual contact, needle stick or transplacental passage (8). Transmission of this viral particle requires the successful interaction of the virus with receptors on the target cell surface (9).

After the HIV-1 particle enters the cell, the nucleoprotein core becomes disrupted. Reverse transcription of the viral RNA genome begins in the cytoplasm and may be completed in the nucleus (10). Interestingly, because of the inaccuracy of reverse transcriptase,

Figure 1: Structure of the HIV-1 virion. The HIV-1 virion contains in its core two single stranded copies of the positive-sense viral RNA genome. This genomic material is associated with repeating units of nucleocapsid protein and is encased within a conical shell composed of a p24 capsid protein lattice. Also contained within the p24 capsid shell are the viral enzymes: integrase and reverse-transcriptase. The p24 capsid is in turn encased within a lipid bilayer taken from the host cell membrane during viral egress. Numerous receptor "spikes" composed of gp120-gp41 glycoprotein hetero-trimers protrude from the virions surface. Figure was obtained with permission from Nature Publishing Group (Permission from the corresponding author as well as the publishing group are depicted in the appendix) (11).

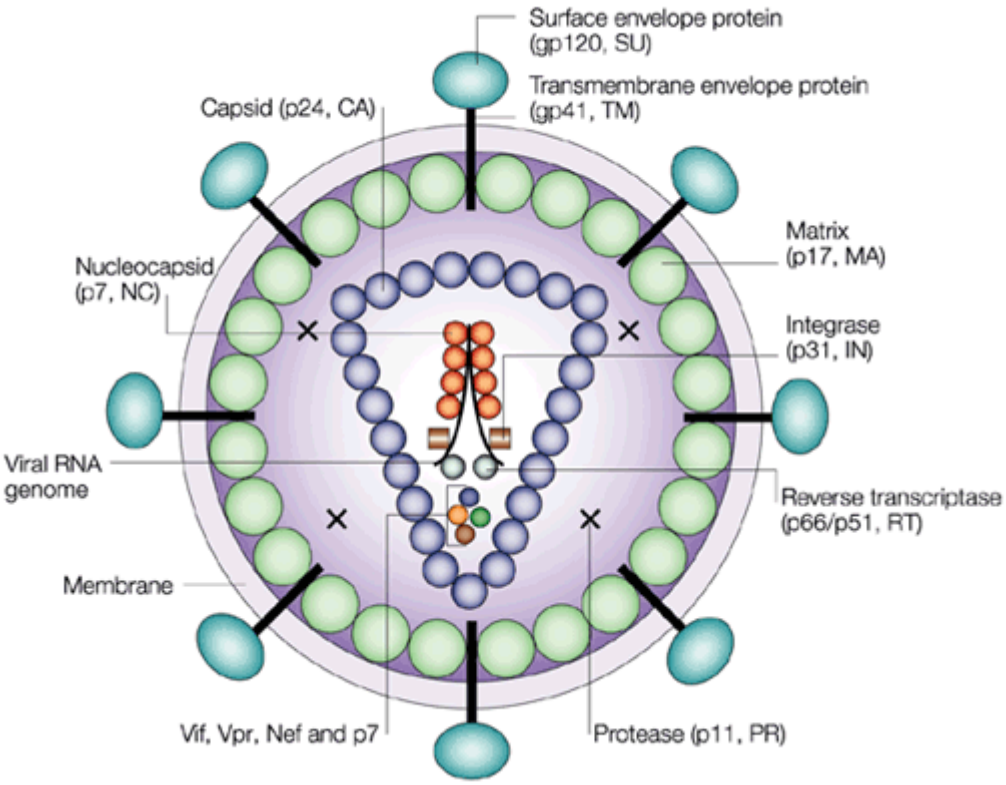
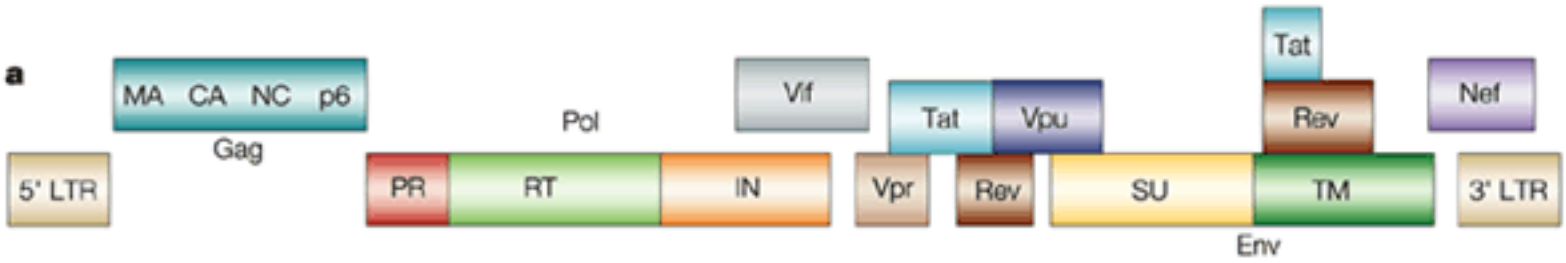

Surface envelope protein
(gp120, SU)
Transmembrane envelope protein
(gp41, TM)
Capsid (p24, CA)
Matrix
(p17, MA)
Nucleocapsid
(p7, NC)
Integrase
(p31, IN)
Viral RNA
genome
Reverse transcriptase
(p66/p51, RT)
Membrane
Vif, Vpr, Nef and p7
Protease (p11, PR)

Figure 2: The HIV-1 genome. The HIV-1 genome codes for three large structural polypeptides (Gag, Pol and Env) and 6 accessory proteins (Vpr, Tat, Vpu, Rev, Nef and Vif). Figure was obtained with permission from Nature Publishing Group (Permission from the corresponding author as well as the publishing group are depicted in the appendix) (11)


a
MA CA NC p6
Gag
Pol
Vif
Tat
Vpu
Tat
Rev
Nef
5' LTR
PR
RT
IN
Vpr
Rev
SU
TM
3' LTR
Env

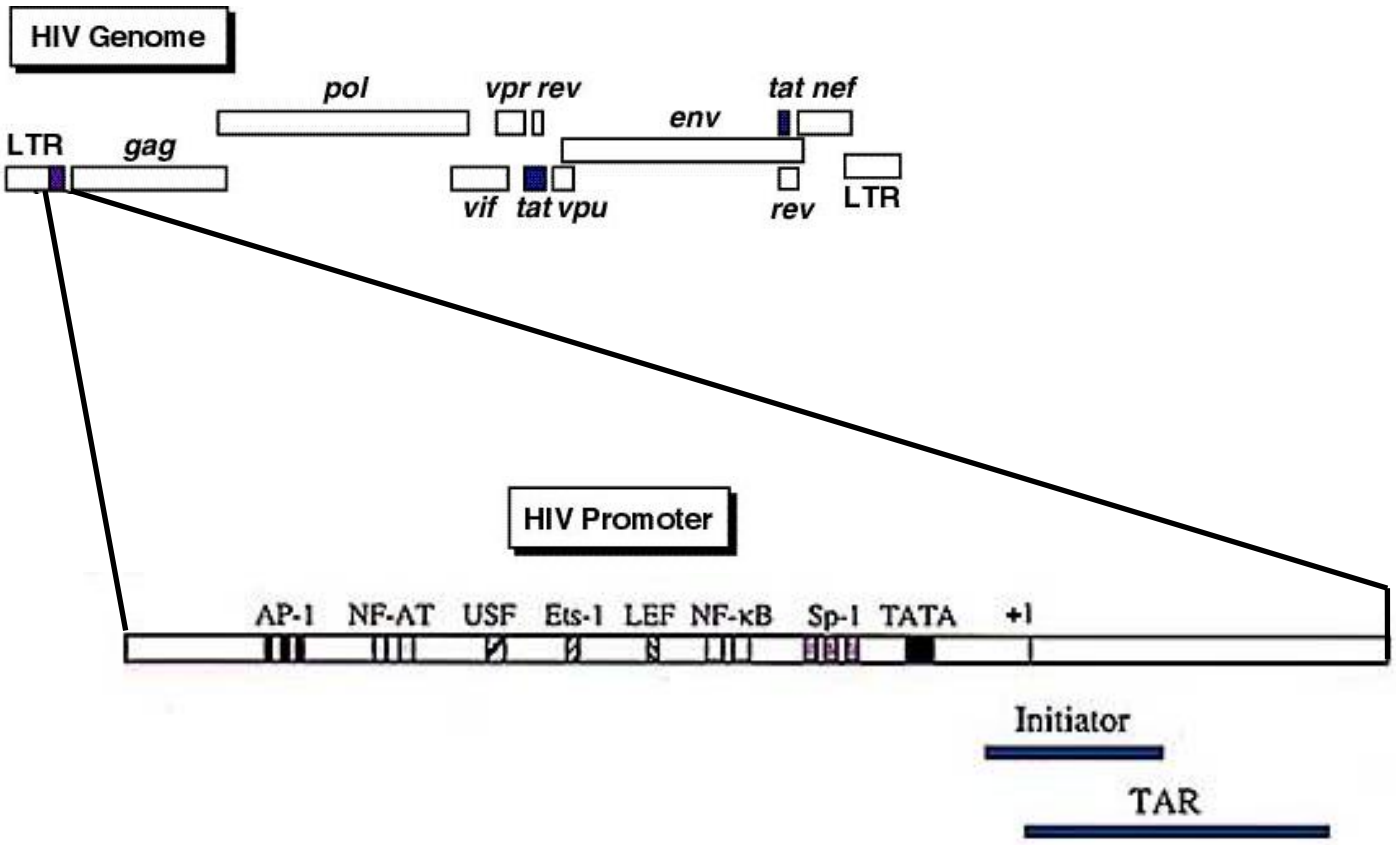
viral clones obtained from single infected individuals differ from each other at the genetic level (12,13). This allows HIV-1 to rapidly diversify in the infected individual and as a consequence, is able to adapt readily to changes in its environment (13).

As with all retroviruses and lentiviruses, HIV-1 must integrate into the DNA of the host cell and to do this it uses HIV-1 integrase (10). As a consequence, the activity of the integrated viral genome, or provirus, is greatly influenced by the metabolic and activation state of the host cell, and the longevity of the provirus is dictated by the life span of the cell that contains it (14,15).

Transcription of the provirus is regulated by sequences known as long terminal repeats (LTR) located immediately upstream and downstream of the viral coding sequences. The LTRs contain polyadenylation signal sequences, TATA box promoter sequence and binding sites for host transcription factors: NF-kB and SP1, see figure 3 (16,17). In the absence of HIV-1 Tat, the basal activity of the LTR varies considerably depending on the integration site, whereas in the presence of Tat, viral expression is efficient regardless of the integration site (18). It is also worth noting that cytokines or other physiologic stimuli to T cells and macrophages are able to enhance viral gene transcription via activation of NF-kB and SP1 (19).

Tat protein acts to enhance the production of complete HIV viral messenger RNA (mRNA) transcripts (20). The synthesis of mature infectious viral particles begins after full-length viral RNA transcripts are produced and the viral genes are expressed as proteins (18). HIV gene expression may be divided into an early stage, during which regulatory genes are expressed, and a late stage during which structural genes are expressed and full-length viral genomes are packaged (7,10).

Figure 3: A schematic representation of the HIV-1 promoter which is located within the 5' LTR of the viral genome. The core promoter consists of the initiator region, the TATA box, and three Sp1 sites. An upstream enhancer contains binding site for several cellular transcription factors including NF-κB, LEF, Ets-1, USF, NF-AT, and AP-1 (16,17). Figure was obtained with permission from Elsevier (Permission from the corresponding author as well as the publishing group are depicted in the appendix) (16).


HIV Genome
pol
vpr rev
tat nef
env
LTR
gag
vif
tat vpu
rev
LTR
HIV Promoter
AP-1
NF-AT
USF
Ets-1
LEF
NF-κB
Sp-1
TATA
+1
Initiator
TAR

After translation of various viral genes transcripts, assembly of infectious particles begins by packaging full-length RNA transcripts within a nucleoprotein complex that includes *gag* core proteins and the *pol* encoded enzymes required for the next cycle of infection (10). This nucleoprotein complex is then enclosed within a membrane envelope and released from the cell by a process of budding from the plasma membrane (10). For full HIV-1 viral life cycle, see figure 4.

1.1.3 Pathogenesis of HIV disease

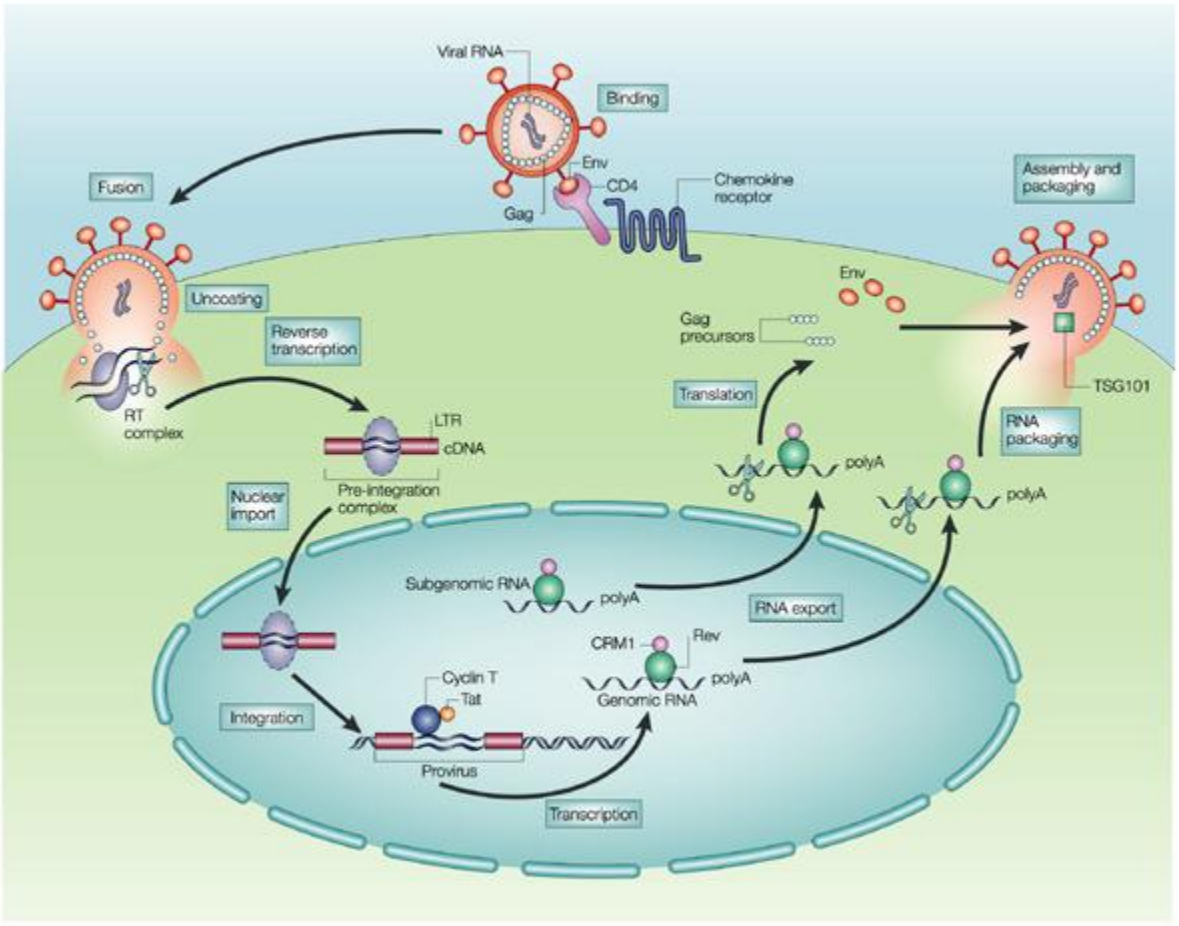
Gradual depletion of CD4 T cell numbers is a classic characterization of HIV infection, leading over the course of years to a loss of immunocompetence, increased incidences of opportunistic infections and increased rates of tumourigenesis (21).

HIV disease begins with acute infection, which is only partly controlled by the adaptive immune response, and advances to chronic progressive infection of peripheral lymphoid tissue (22). The subsequent events in the infection can be divided into several phases: acute infection followed by a chronic infection, also referred to as the clinical latency period, and finally symptoms of AIDS.

1.2 Immune response mounted against HIV-1

HIV-specific humoral and cell-mediated immune responses develop following infection but generally provide limited protection. The early response to HIV infection is, in fact, similar in many ways to the immune response to other viruses and which attempts to clear most of the virus present in the blood and in circulating T cells. Nonetheless, it is clear that these immune responses fail to eradicate all viruses and the infection, if untreated, eventually overwhelms the immune system in most individuals (23).

Figure 4: The HIV-1 cellular life cycle. **1)** HIV-1 particles bind to the host cell via interactions between the gp120 surface spike and CD4. **2)** gp120 interactions with the CCR5 or CXCR4 co-receptors results in viral uncoating. **3)** Viral RNA is transcribed in the cytoplasm into viral DNA by the viral reverse transcriptase. **4) V**iral DNA then enters the nucleus and integrates into the host genome using viral integrase. **5)** Viral RNAs and proteins are transcribed and **6)** translated, respectively, by host machinery. The viral glycoproteins are translated in the ER and processed by host machinery to produce the mature gp120 and gp41 subunits. Other viral proteins are translated in the cytoplasm. **7)** Viral structural proteins congregate at the inner leaflet of the cell membrane and assemble into immature virions. **8)** Virions derive their envelope from the host lipid bilayer and bud directly off the cell membrane. **9)** Once free of the cell, HIV-1 proteases packaged into the immature virions cleave numerous polypeptides to yield the mature virion. Figure was obtained with permission from Nature Publishing Group (Permission from the corresponding author as well as the publishing group are depicted in the appendix) (24).



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The initial adaptive immune response to HIV infection is characterized by expansion of CD8 T cells specific for HIV peptides. As many as 10% or more of circulating CD8 T cells may be specific for HIV during the early stages of infection (25). These cytotoxic T lymphocytes (CTLs) control infection in the acute phase, but ultimately prove ineffective because of the emergence of viral escapes mutants (variants with mutated antigens). Although CD4 T cells also respond to the virus, the importance of this response is unclear.

1.2.1 Adaptive Immune response

The defining characteristics of adaptive immunity are exquisite specificity for distinct molecules and the ability to 'remember' and respond more vigorously to repeated exposures to the same microbe. It addition, adaptive immunity has an extraordinary capacity to distinguish between different, even closely related, microbes and molecules, and for this reason it is also called 'specific immunity'. The two types of adaptive immune responses are humoral immunity and cell-mediated immunity which function to eliminate different types of microbes.

In the context of a viral infection, the cell mediated immune response is initiated when antigen presenting cells (APCs) such as dendritic cells residing at the initial sites of infection take up viral antigens, become activated, and migrate to regional lymph nodes. There, the APC display viral peptide bound to MHC II molecules to naïve CD4 T cells through engagement of antigen specific surface T cell receptors (TCRs) as well as the CD4 co-receptor. Following recognition of viral antigen in the context of MHC class II, a cascade of signaling events is initiated within the CD4 T cell which results in activation, proliferation, and differentiation into a T helper 1 (Th1) phenotype. These activated CD4 T cells secrete a variety of cytokines such as

IL-2, tumour necrosis factor (TNF)-β and interferon (IFN)-γ which support the differentiation and cytotoxic activity of CD8 T cells.

CD8 T cells become activated following engagement with a virally infected target cell or APCs displaying an MHC I complex. In the context of proper secondary signals (i.e. CD80, CD86) and local cytokine environment, these CD8 T cells undergo clonal expansion and differentiate into effector CD8 T cells. Effector CD8 T cells traffic out of the lymph node and into peripheral circulation, homing to the site of infection. Effector CD8 T cells are commonly referred to as CTLs, which emphasizes their ability to kill virally infected target cells. CTLs are potent antiviral effector cells due to their ability to produce both inflammatory mediators as well as cytotoxic effector molecules.

Effector CD8 T cells identify infected cells through recognition of foreign antigens displayed on the infected cell's surface in the context of an MHC I complex via their TCR and CD8 co-receptor. The infected cell is targeted and its death occurs during the next 2 to 6 hours and proceeds even if the effector CD8 T cell detaches. The cell-to-cell contact creates an 'immunological synapse' which allows for delivery of cytoplasmic granules triggering target cell apoptosis. These granules include granzyme A which promotes DNA damage by cleaving histone H1 (26) and granzyme B which enters the cytosol to initiate apoptotic cascades by cleaving pro-caspase 3 (27) and induce the permeabilization of mitochondrial membranes (28). The granules also include perforin, a membrane-perturbing molecule homologous to C9 complement protein, and a sulfated proteoglycan, serglycin, which serves to assemble a complex containing granzymes and perforin. Both perforin and granzymes are required for

efficient effector CD8 T cell killing of target cells, and it is known that perforin's main function is to facilitate delivery of the granzymes into the cytosol of the target cell.

In addition to cytoplasmic granules, effector CD8 T cells induce target cell apoptosis via engagement of Fas trimer complexes found on the surface of their target cells with the Fas ligand (FasL) on the effector CD8 T cells. This interaction results in the extrinsic apoptotic cascade via recruitment of Fas-associated death domain (FADD) proteins. This initiates caspase cascades resulting in DNA cleavage and cytochrome C release from the mitochondria (29,30).

After delivering the 'lethal hit', the effector CD8 T cell detaches from the target cell and is freed to identify another target cell. In this manner, activated cytotoxic CD8 T cells act as "serial killers"; each cytotoxic CD8 T cell killing numerous target cells.

Ideally, the immune response mentioned above overwhelms the viral infection and results in the complete eradication of the virus from the host. Following viral eradication, the expanded pool of effector T cells does not remain constitutively activated. Instead, during the *downregulation phase*, the majority (>90%) of the virus-specific T cells present at the peak of the immune response dies by apoptosis. The remaining 5–10% of T cells survives through the *contraction phase* and constitutes a long-lived pool of memory T cells. In this way, a beneficial memory of past infections is established as, by comparison with naïve hosts, an increased number of virus-specific T cells are maintained which are tuned to rapidly respond if they re-encounter cells infected with the same pathogen.

1.2.2 CD 8 T cells in HIV infection

1.2.2.1 CD8 T cell immune response

After the first week of infection viral-specific CD8 T cells form one of the cornerstones of immune control for HIV-1. CTL response initially follows the rise in HIV in the blood and when that response reaches a peak the virus level falls; after that there is an inverse relationship between CTL response and viral load (31). Following the peak in CD8 T cell response, the virus sequence starts to change drastically (32). Rapid selection of mutations occurs at discrete sites in the virus genome as viramia declines to the viral set point (32,33). CTLs recognize virus peptides presented by human leukocyte antigen (HLA) class I molecules, and different HLA types present different peptides and thus affect the quality of the immune response (34). The HLA alleles HLA-B*5701, HLA-B*5703, HLA-B*5801, HLA-B27 and HLA-B51 are all associated with good control of the virus and a slower progression to AIDS (35), partly because the epitopes recognized by the T cells in these individuals are focused on conserved regions of the viral Gag protein (34).

1.2.2.2 CD8 T cell dysfunction

Impaired cell-mediated immunity and inefficient immunologic control of viral replication is evident in HIV^+ patients with progressive disease (36-39). Although CD8 T cells do not become depleted like their $CD4^+$ counterparts (40); it is clear that HIV-1 directly affects CD8 T cell function. CD8 T cells begin to display impaired function before CD4 T cells become depleted to an appreciable extent (39,41,42). The impairment of CD8 T cells isolated from $HIV\text{-}1^+$ individuals includes but is not limited to the following features: aberrant surface marker phenotypes, decrease in cytolytic activity, and decrease in antigen induced clonal expansion (43). Furthermore, the CD8 T cells show low perforin expression in lymph nodes of HIV^+ persons (44).

1.3 Interleukin-7

Interleukin-7 like other cytokines is a pleiotropic immune regulatory protein that plays an important role in the normal development and maintenance of the human immune system (45). IL-7 originally named lymphopoietin-1, was first identified by a group of investigators at the Immunex Research and Development Corporation in 1988 as a B cell growth factor (46,47). While IL-7 shares pro-survival and proliferative capacities with related cytokines, including other interleukin family members, it has been shown to play non-redundant roles in the development and homestasis of T and B cells in mice (48,49) and in T cells in humans (50). IL-7 has a role in V(D)J recombination in developing T and B cells (51,52), differentiation of CD8 T cells in the thymus (53), induction of proliferation of T cells in the periphery (54,55) and establishment of T cell memory (56,57) .

The active form of human IL-7 is a glycoprotein of 25 kDa (58) and is encoded by the IL-7 gene on chromosome 8 (locus 8q12-q13) (46). IL-7 was considered to be expressed constitutively and that T cells competed for limiting amounts of this essential cytokine (59). However, this has turned out not to be the case and IL-7 is now recognized as an acute-phase cell-signaling molecule (60). IL-7 is expressed in the stroma of lymphoid tissue and some nonlymphoid tissues including kidney, skin, intestines, uterus, brain and adult liver (Table 1). Circulating levels of IL-7 in sera of healthy individuals are relatively low ranging from 0.3-8 pg/ml (61,62). IL-7 may also be sequestered at the cell surface by heparan sulfate and fibronectin which may protect it from proteolytic cleavage and increase its availability to neighboring target cells (63,64).

Table 1: Cells and Tissue That Produce IL-7. Figure was obtained with permission from Kluwer Academic (Permission from the corresponding publishing group are depicted in the appendix) (65).

IL-7 producer	Protein	mRNA	Ref.
Bone marrow stromal cells	+	+	(47,66,67)
Fetal liver stromal cells	n.d.	+(m)	(68)
Spleen	n.d.	+(m,h)	(46,69)
Fetal and adult thymus	+	+(m,h)	(46,69,70)
Thymic stromal cells	+	+	(71,72)
Kindey tissue	n.d.	+	(73)
Keratinocytes	+	+	(74)
Intestine epithelial cells	+(h)	+	(71,75)
Lymph node T zone fibroblastic reticular cells	+	+	(76)
Peripheral blood dendritic cells	+	+	(77)
Follicular dendritic cells	+	+	(57,78)
Endothelial cells	+	+	(57)
Smooth muscle cells	+	+	(57)

n.d.. Not done; m, mouse; h, human; r, rat.

1.3.1 Interleukin-7 in HIV infection

Increased levels of circulating IL-7 were observed during HIV infection (average 18 pg/mL (79,80)) along with a downregulation of IL-7 receptor α-chain (IL7Rα) expression on T cells (62,81-89). There has been considerable debate over the causes and consequences of these observations. An inverse relationship between plasma levels of IL-7 and CD4 T cell counts was found in HIV^+ persons, thus suggesting that increased production of IL-7 represents a homeostatic response to HIV-associated CD4 T cell depletion (90). Unfortunately, these increased levels of IL-7 are not sufficient to maintain T cell homeostasis, since in the absence of antiretroviral therapy most HIV^+ persons will develop progressive CD4 T cell decline and clinical signs of AIDS (90). An alternative hypothesis to explain the inverse relationship between IL-7 levels and CD4 T cell counts is that increased levels of this cytokine result from decreased numbers of circulating $IL7R\alpha^+$ T cells resulting in reduced consumption of the cytokine (45). Unfortunately, this hypothesis does not take into account all the other cell types (macrophages and endothelial cells) that also express CD127 and are not reduced in HIV infection.

1.4 Interleukin-7 Receptor

The IL-7 receptor consists of two components, the IL-7Rα chain (CD127) and the common γ-chain (CD132) which is shared by receptors for IL-2, IL-4, IL-9, IL-15 and IL-21. Both receptor components are members of the cytokine receptor family. Each of these chains is expressed on the surface independently of each other, although the two chains could pre-associate as shown for several other types of cytokine receptors. The essential roles of common ɣ- chain cytokines play in T cell differentiation, maturation and proliferation have been extensively studied in mice where ɣ-chain knockout mice demonstrate a complete lack of or at least siginificant reduction

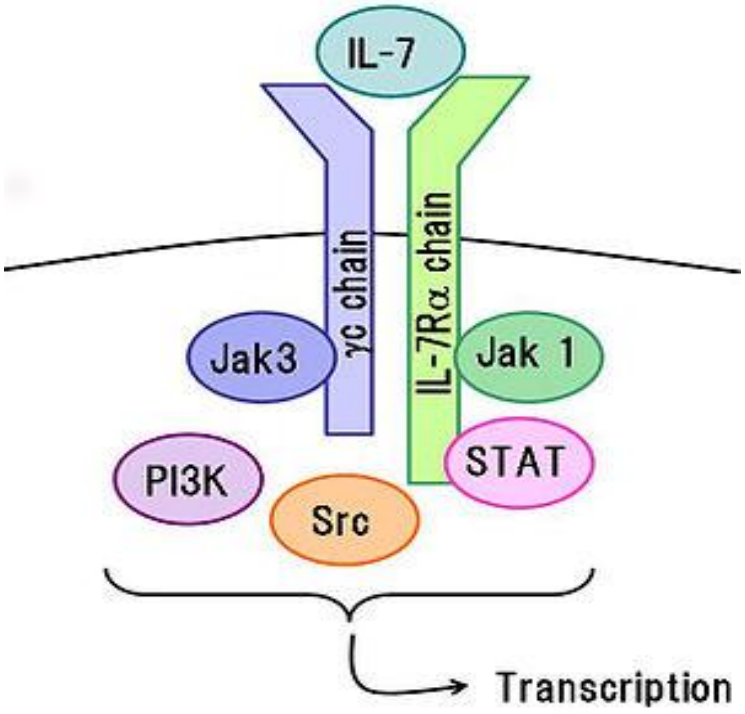
in number of NK, B and T-cells, reviewed in (91). In fact, mutations in CD127 (92) and CD132(93,94) genes cause severe combined immune deficiency syndrome (SCID) in humans and in mice.

IL-7 signalling in human peripheral blood lymphocytes is mediated by low affinity binding (K_d= 100 nM) and high affinity binding (K_d= 65 pM) (58). IL-7 binding to CD127 promotes dimerization with CD132 thus forming the IL-7 receptor complex and activating signal transduction (95). C127 and CD132 associated through their cytoplasmic tails with Janus Kinase 1 (JAK1) and JAK3 respectively, see figure 5 (96-98).

1.4.1 Regulation and Expression

The expression of CD127 is strictly regulated during the development of T cells. In addition, CD127 expression by mature T cells is dramatically influenced by extrinsic stimuli, including antigens and cytokines (99). The amount of CD127 expressed by a lymphocyte could have two effects. First, there could be an effect intrinsic to the lymphocyte: that is, the more receptor a cell expresses, the more signals it should receive for survival and proliferation (99). Second, there could be an effect extrinsic to the lymphocyte: that is, the more receptor a cell expresses, the more IL-7 it could consume; thereby depriving neighbouring cells of their survival and proliferative signals (99). After encountering IL-7, T cells transiently downregulate CD127 expression; it has been proposed that this downregulation is altruistic as, having satisfied its IL-7 requirement, the T cell would stop needlessly consuming IL-7 (100,101). Naïve T cells and both central and effector memory cells express high levels of CD127 (59,102). Dendritic cells,

Figure 5: IL-7 receptor and signalling. The IL-7 receptor α-chain, CD127, is associated with JAK1 and the common γ chain, CD132, is associated with JAK3. http://en.wikipedia.org/wiki/File:IL-7receptor_and_signaling.jpg


IL-7
γc chain
IL-7Rα chain
Jak3
Jak 1
PI3K
Src
STAT
Transcription

monocytes and bone-marrow derived macrophages also express CD127 but at a lower levels compared to naïve and memory T cells (59,103).

Without stimulation, CD127 constitutively recycles on and off the cell membrane through the early endosomal system with a half-life of around 55 hours (104). The majority of CD127 returns to the surface while a small percentage of CD127 is shunted to the lysosome for degradation (104). Degraded protein is replaced by *de novo* synthesis and surface CD127 levels remain relatively constant (104).

CD127 is hypothesized to get downregulated in T cells by different mechanisms at different stages of development and in different subpopulations; this has been proposed by some to have a remarkable altruistic function, as noted earlier (100). TCR signalling also downregulates CD127 expression at the level of transcription (51,105,106), providing an additional layer of intricacy to IL-7: TCR crosstalk. CD127 transcripts can be suppressed by other members of the γ-chain family of cytokines such as IL-2, IL-4, and IL-15, as well as the pro-inflammatory cytokine IL-6 (100).Perhaps the most important regulator of CD127 expression is IL-7 itself. We and others have shown that IL-7 signalling downregulates surface CD127 protein, directing it to the proteasome for degradation (82,83,104,107-110). The cellular mechanisms responsible for CD127 downregulation from the surface of cells in response to IL-7 have yet to be completely elucidated, although several of the critical components have been identified. IL-7 binding to CD127 initiates rapid internalization of surface CD127 protein and activation of JAK which in turn phosphorylates Y449 in the cytoplasmic tail of CD127 as well as STAT5. Phospho-STAT5 dimers translocate to the cell nucleus where they up regulate the expression of suppressor of cytokine signalling (SOCS) 1, SOCS 2 and cytokine-inducible SH2 containing (CIS)

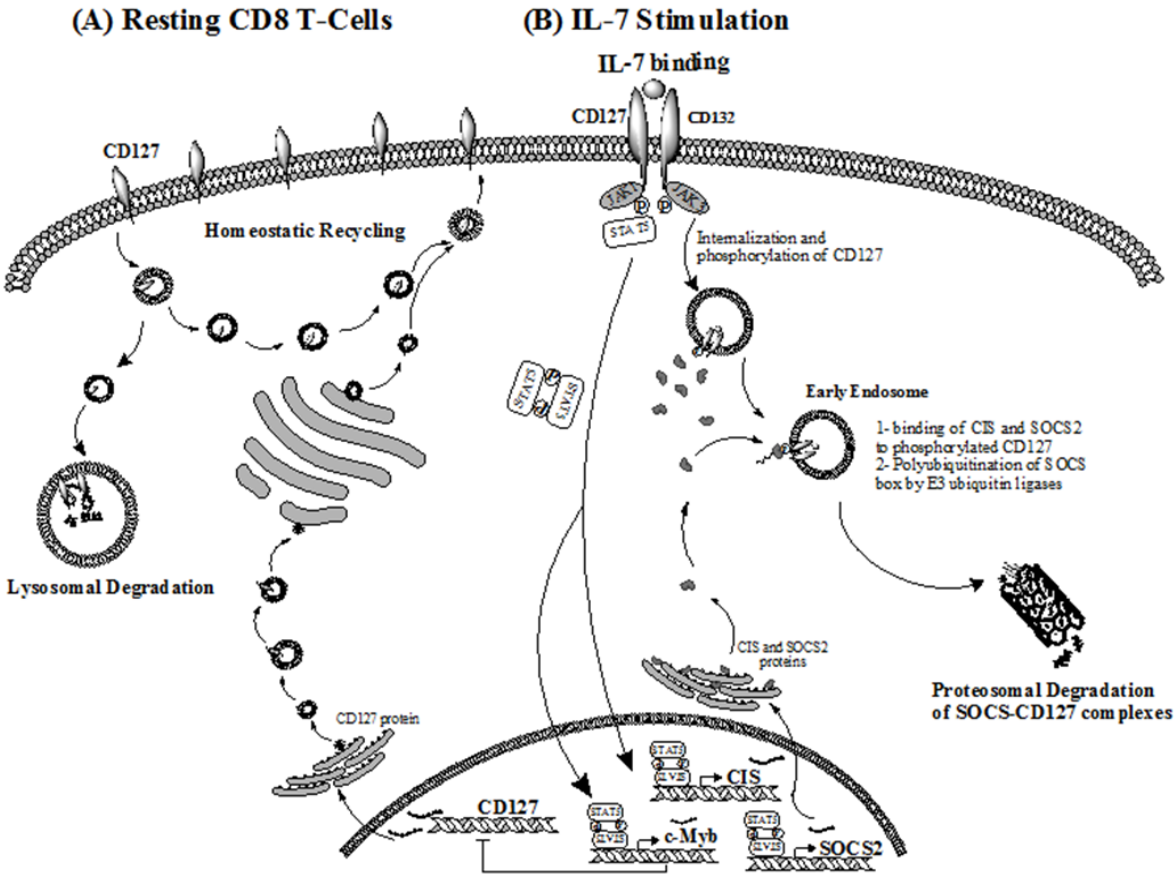
protein (111). CIS and SOCS2 then bind to the phosphorylated tail of CD127 in early endosomes and likely recruit an E3 ubiquitin ligase shuttling the complex through late endosomes to the proteasome for degradation. See figure 6 for model illustrating homeostatic recycling of CD127 following IL-7 stimulation.

At lower concentrations of IL-7, CD127 surface protein is downregulated from the surface of T cells in a transient manner. It was suggested that IL-7 can induce the internalization of CD127 transiently through a JAK independent mechanism (112). Here, primary human T cells pre-treatment with JAK inhibitor followed by IL-7 treatment resulted in the removal of surface CD127 over the course of six hours which then recovered by 24 hours, without any changes in whole cell CD127 protein level (112). At higher IL-7 concentrations, CD127 downregulation is sustained for prolonged periods with CD127 gene transcription being also shut off to inhibit the production of *de novo* protein (111). Here, the downregulation of CD127 is depended on the activity of JAK and is achieved via the recruitment SOCS2 and CIS (111). This observation is in line with previous work demonstrating that SOCS proteins directly inhibit Jak1-mediated signalling (113), as well as recruit cellular E3 ligases to the cytosolic tails of numerous receptors inducing their ubiquitination and subsequent proteasomal degradation (114-117).

1.4.2 CD127 Structure

IL-7Rα belongs to the cytokine receptor homology class I (CRH1) family and is composed of three domains: extracellular, transmembrane and intracellular. The full length form of CD127 consists of 439 amino acids (aa), with a calculated molecular weight 49.5 kDa (58). However, in

Figure 6. Cumulative model illustrating homeostatic recycling of CD127 at the cell surface and down regulation of CD127 surface protein and gene transcription following IL-7 stimulation. In resting CD8 T cells, CD127 recycles on and off the cell membrane likely through early endosomes with basal protein turn over in the lysosome. IL-7 binding to CD127 initiates rapid internalization of surface CD127 protein and activation of JAK which in turn phosphorylates Y449 in the cytoplasmic tail of CD127 as well as STAT5. Phospho-STAT5 dimers translocate to the cell nucleus where they up regulate the expression of CIS, SOCS1 and SOCS2 proteins. CIS and SOCS2 then bind to the phosphorylated tail of CD127 in early endosomes and likely recruit an E3 ubiquitin ligase shuttling the complex through late endosomes to the proteasome for degradation. In parallel, activated STAT5 dimers induce c-Myb expression which in turn binds to the CD127 gene promoter and suppresses CD127 transcription (111). Figure was obtained with permission from author (Permission from the corresponding author as is depicted in the appendix) (111)**.**


(A) Resting CD8 T-Cells
(B) IL-7 Stimulation
IL-7 binding
CD127
CD132
CD127
Homeostatic Recycling
JAK1
JAK3
P
P
STATS
Internalization and
phosphorylation of CD127
Early Endosome
1- binding of CIS and SOCS2
to phosphorylated CD127
2- Polyubiquitination of SOCS
box by E3 ubiquitin ligases
Lysosomal Degradation
CIS and SOCS2
proteins
Proteosomal Degradation
of SOCS-CD127 complexes
CD127 protein
CD127
STAT5
DVIS
CIS
c-Myb
SOCS2

SDS-PAGE under reducing conditions, the apparent molecular mass of CD127 is approximately 75-90 kDa due to extensive glycosylation.

The protein has a structure typical for binding an α-helical cytokine, and it is composed of a 219 aa extracellular domain, a 25 aa transmembrane domain, and a 195 aa intracellular domain (118). Structurally, the extracellular domain shares homology with other members of the type I family of cytokine receptors. In particular four invariant cysteine residues are located at the N-terminus of the extracellular domain, which are involved in intra-chain disulfide bond formation (119). Close to the transmembrane domain, the extracellular domain contains a Trp-Ser-Trp-Ser (WSXWS) motif involved in the proper folding of the protein. Finally the extracellular domain contains two fibronectin type III-like modules connected by a 3_{10}-helical linker (120). The CD127 extracellular domain also contains six N-linked glycosylation sites (119). Although they do not occur in the IL-7 binding site, glycosylation significantly enhances IL-7 binding to the receptor by up to 300-fold. It appears glycosylation of CD127 stabilizes its interaction with CD132 which in turn stabilizes receptors: ligand binding (121).

1.4.3 Functional regions of the IL-7R intracellular domain

The CD127 intracellular domain is fundamental for signal transduction. While it lacks intrinsic kinase activity, the intracellular domain contains several structural and functional motifs that are involved in recruitment of signal transducing molecules. This 195 aa domain contains four characterized regions. A small membrane-proximal domain, Box 1, conserved in mouse and humans, is proline rich and is important for the binding of Janus family tyrosine kinases (JAKs) (122). Next to Box 1 is an acidic domain, followed by a serine-rich domain, and a

C-terminal tyrosine domain that contains three tyrosine residues also conserved in mouse and humans (Y401, Y449 and Y456) (118). See regions of CD127 in figure 7.

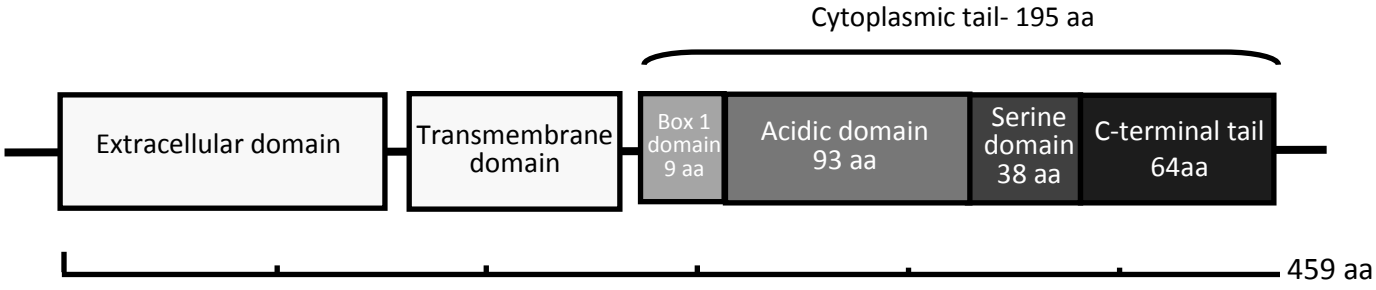
1.4.3.1 Box I/ Y449 (JAK/STAT related region)

The cross-specificity in JAK and STAT activation results in one of the major nodes of cytokine signalling crosstalk. IL-7 was shown to induce a rapid and dose-dependent tyrosine phosphorylation of JAK1 and JAK3 (123). Furthermore, JAK1 and JAK3 knockout mice display severely impaired thymic development, suggesting their importance in IL-7 signalling (reviewed in (58)). JAK1 is known to associate with CD127 Box 1. In support of this, JAK1 phosphorylation was shown to be eliminated when CD127 Box1 was deleted (95). Most literature indicates JAK3 is pre-associated uniquely with the intracellular domain of CD132 (reviewed in (124)).

IL-7 was also shown to induce a rapid and dose-dependent phosphorylation of STAT5a/b leading to DNA-binding activity (123). STAT5 phosphorylation was eliminated with a point mutation of Y449 on CD127, which is consistent with a previous study showing the direct association of SH2 domain of STAT5 with phosphorylated Y449 on CD127 (125). STAT is known to dock on phosphorylated tyrosine residues and after being itself phosphorylated, it mediates dimerization, interacting with other SH2 domains (58).

In this model of IL-7 signalling, IL-7 binds to CD127 and then recruits CD132, bringing together their intracellular domains bearing JAK1 and JAK3. These two kinases have a low intrinsic enzymatic activity, but after their mutual phosphorylation, they acquire much higher activity. JAK1 in turn phosphorylates the critical Y449 site on CD127 (95). Phosphorylated Y449 serves as a docking site for STAT5 and possibly other adaptors, and they would in turn become phosphorylated by JAK1 and JAK3. Phosphorylated STAT5 dimers translocate to the nucleus and

Figure 7. Linear representation of wild type CD127. Note this representation is not to scale.


Cytoplasmic tail- 195 aa
Extracellular domain
Transmembrane domain
Box 1 domain 9 aa
Acidic domain 93 aa
Serine domain 38 aa
C-terminal tail 64aa
459 aa

trigger the transcriptional events of their target genes (reviewed in (58)). Perforin and B-cell lymphoma 2 (BCL-2) are two of the many genes unregulated through STAT5 signalling (126). During thymopoiesis, STAT5 association with the TCR promoter has been implicated in proper V(D)J rearrangement through histone acetylation (127).

1.4.3.2 Y449 (PI3K related region)

IL-7 has clearly been shown to also activate PI3 kinase in human T cells (128) where JAK3 was found to associate, phosphorylate and activate the catalytic p85 subunit of PI3 kinase (128-130). The p85 subunit was also shown to directly bind to phosphorylated Y449 via an SH2 domain which then would activate the catalytic subunit (131). AKT/protein kinase B (PKB) is a downstream target of the PI3 kinase and plays important roles in glucose metabolism, cell proliferation and apoptosis (132). PI3 kinase dependent IL-7 signalling also regulates the expression of cyclin-dependent kinases (Cdks) which drive proliferation (133), sequester the pro-apoptotic molecules BAD (BCL-2-antagonist of cell death) and BAX (BCL-2-associated X protein) (95,134), and induce the translocation of GLUT-1 to the cell membrane (135,136).

1.4.3.3 Acidic domain (Src family related region)

IL-7 stimulation leads to the activation of Src family of kinases in some T lymphoblastic cell lines (137) and human primary T cells (138). In T lymphoblastic cell lines, $p59^{fyn}$ has been shown to associate with the acidic domain of CD127 (139). In human T cells, both $p59^{fyn}$ and $p56^{lck}$ were shown to be physically associated with CD127 (138). The effects of these associations and their relevance in humans *in vivo* are not yet clear.

1.4.3.4 Serine domain

The serine domain was shown to be dispensable for T cell survival and development in vivo (95).

1.4.4 Signal transduction pathways

IL-7 signaling is thought to function via the classical stepwise cytokine-induced receptor heterodimerization paradigm (reviewed in (140,141,141)). In this mechanism, IL-7 interacts with the CD127 extracellular domain, forming a 1:1 assembly (141,142), which subsequently recruits CD132, producing the signaling complex. The association of the two receptors by IL-7 brings JAK1 and JAK3 together which mutually phosphorylate each other, increasing their kinase activity. JAK3 then phosphorylate Y449, creating a docking site for several signalling molecules including STAT5 and PI3 kinase. Activation of JAK-STAT and PI3 kinase are considered the major signaling pathways in T cells (58). IL-7 has been shown to associate with a variety of other signaling molecules including $p56^{lck}$, $p59^{fyn}$, p38 and members of the Src family of kinases; however their relevance is not yet clear (138,139).

IL-7 signalling is important for both thmyocytes and mature lymphocytes leading to proliferation, cell survival and differentiation. One significant consequence of IL-7 receptor signalling is the maintenance of cell survival by promoting a favourable balance of BCL-2 family members by increasing expression of the survival proteins BCL-2 and MCL1 (myeloid-cell leukaemia sequence 1), and by redistributing the cell-death proteins BAX and BAD (reviewed in (58)), see figure 8. In thymocytes, IL-7 signalling provides trophic and proliferative signals to double-negative thymocytes and also directly instructs recombination of the T cell receptor γ-chain (TCRγ) locus (51,52), see figure 8.

1.4.5 CD127 Dysregulation in HIV Infection

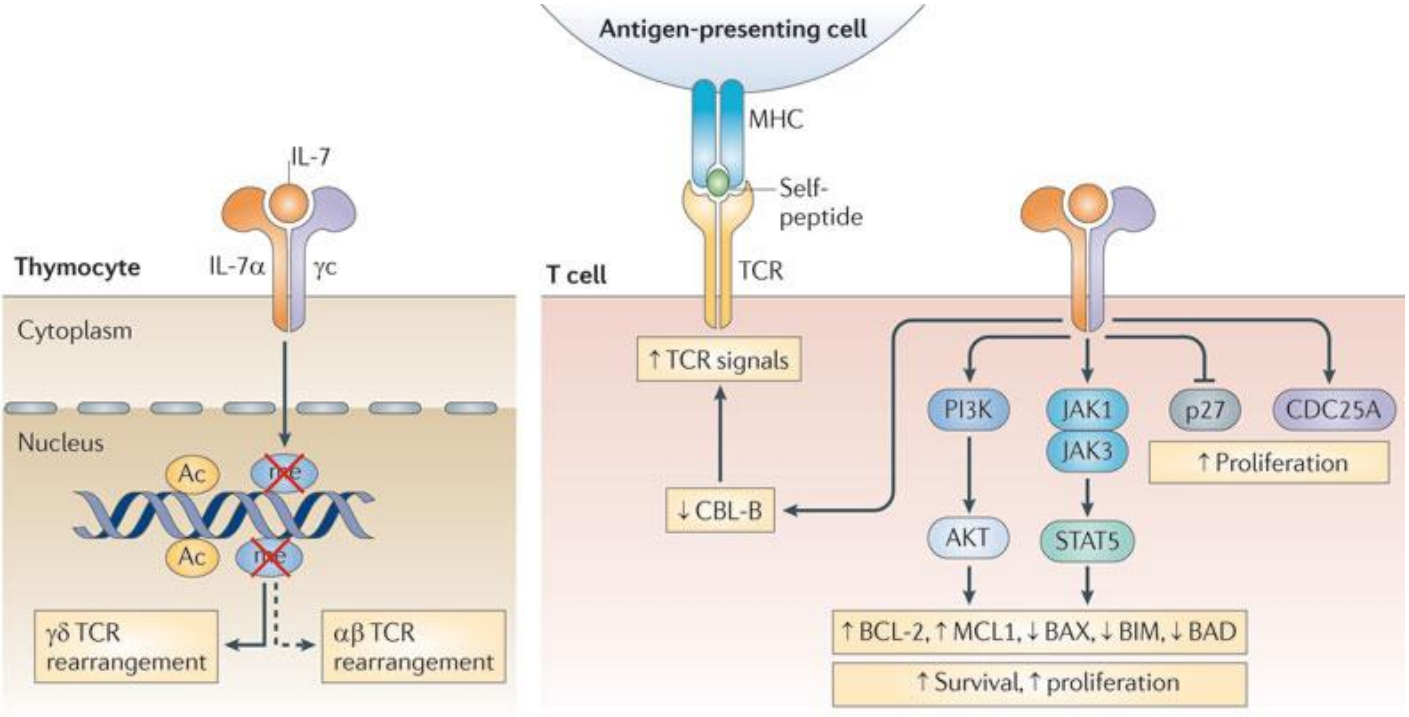
We and others have demonstrated that in HIV^+ persons with uncontrolled plasma viremia, significantly fewer $CD8^+$ T cells express cell surface CD127 compared to healthy individuals (82,84,86,143,144). The progressive reduction in the fraction of CD8+CD127+ T cell subset often drops below 50% in peripheral T cells (80,82,84,85,144). The CD8+CD127- cells do not express CCR7 or CD62L and produce IFNγ, but not IL-2 (85). Of note, the levels of CD8+CD127- T cells in HIV^+ persons correlated directly with the main markers of disease progression (i.e. plasma viremia and CD4+ T cell count), as well as with the indices of overall T cell activation (85). Fortunately, suppression of viral replication with effective antiretroviral therapy is associated with a greater proportion of $CD8^+$ T cells expressing CD127 compared to untreated HIV^+ persons (82,85,143).

1.5 HIV-1 Tat

1.5.1 Function of HIV Tat protein

HIV-1 encodes small nuclear transcriptional activator protein known as *trans*-activator of transcription (Tat) (146). Unlike other transactivtors, which only bind to DNA, HIV-Tat can bind to HIV proviral DNA (147), host cell DNA (148) and viral RNA and affect transcription. The ability of Tat to bind to viral RNA during its transcription distinguishes it from other transactivators. Tat is the first protein to be produced after cDNA synthesis by reverse transcriptase (149). It is not only required for initiation of viral transcription but indispensable for full-length RNA synthesis (150). The rate of transcription of HIV-1 provirus varies greatly. It is very low in the early phases of HIV infection but increases hundreds of folds during late phases of infection (151). Sodroski *et al.* showed that induction or transactivation of transcription is

Figure 8. Interleukin-7 receptor signalling cascades. IL-7 signals through the IL-7 receptor, a heterodimer comprised of CD127 and CD132. During T cell development in the thymus, IL-7-mediated signalling participates in TCR gene rearrangement through DNA demethylation and histone acetylation. In all T cells, IL-7-mediated signalling initiates downstream signalling pathways through JAK1, JAK3 and PI3 kinase, resulting in the phosphorylation and activation of STAT5. These result in changes in the expression of BCL-2 family members, such as increased expression of the anti-apoptotic molecules BCL-2 and MCL1 and decreased expression of the pro-apoptotic molecules BAX, BIM and BAD. IL-7-mediated signalling also leads to decreased levels of $p27^{Kip1}$, increased levels of CDC25A (cell division cycle 25 homologue A) and changes in the expression of TCR modulators such as Casitas B-lineage lymphoma B (CBL-B). The result of IL-7-mediated signalling is increased T cell survival, increased proliferation, augmented TCR signals and, for recent thymic emigrants, TCR-independent proliferation. Figure was obtained with permission from Nature Publishing Group (Permission from the corresponding author as well as the publishing group are depicted in the appendix) (145).


Antigen-presenting cell
MHC
Self-peptide
TCR
T cell
IL-7
Thymocyte
IL-7α
γc
Cytoplasm
Nucleus
Ac
me
Ac
me
γδ TCR rearrangement
αβ TCR rearrangement
↑ TCR signals
↓ CBL-B
PI3K
JAK1
JAK3
p27
CDC25A
↑ Proliferation
AKT
STAT5
↑ BCL-2, ↑ MCL1, ↓ BAX, ↓ BIM, ↓ BAD
↑ Survival, ↑ proliferation

due to a HIV-Tat protein (152). *In vitro* studies have shown that, only short transcripts are synthesized in absence of Tat protein and most of the full-length transcripts are synthesized in the presence of HIV-Tat protein (153).

Tat increases the transcriptional rate in three different ways. First, Tat modifies chromatin conformation at the proviral integration site and makes it more suitable to viral transcription. Tat binds to a secondary hairpin structure formed at the 5' end of newly formed HIV RNA transcripts, termed the trans-activation response (TAR) element, via cyclin T1 bridging the activation domain of Tat and the TAR loop, see figure 9 (reviewed in references (151,154,155). Through this interaction, Tat recruits a series of transcriptional complexes, including enzymes with histone and factor acetyl transferase (HAT and FAT) activities, which modify chromatin and increase transcription (156). Tat allows for a several fold increase in the rate of HIV transcription after binding to TAR (157). With Tat protein, long polyadenyhlated RNA and increased gene expression is ensured (158-161).

Second, Tat recruits positive transcriptional elongation factor (pTEFb) to adjust the activity of RNA polymerase II (RNAPII). In mammalian cells, RNAPII activity is controlled by the phosphorylation status of its carboyxyl-terminal domain (CTD) (162). Hyperphosorylation increases the processivity of the enzyme complex, while the hypophosorylation correlates with low processivity (163). In the absence of Tat, transcription from HIV-1 LTR produces predominantly short RNA transcripts because hypophosphorylated RNAPII is arrested prematurely following the actions of negative elongation factors, including DSIF (5,6-dichloro-1-beta-D-ribofuranosylbenzimidazolesensitivity-inducing factor) and NELF (negative elongation factor complex) (164). pTEFb, a kinase complex capable of phosphorylating the CTD of RNAPII,

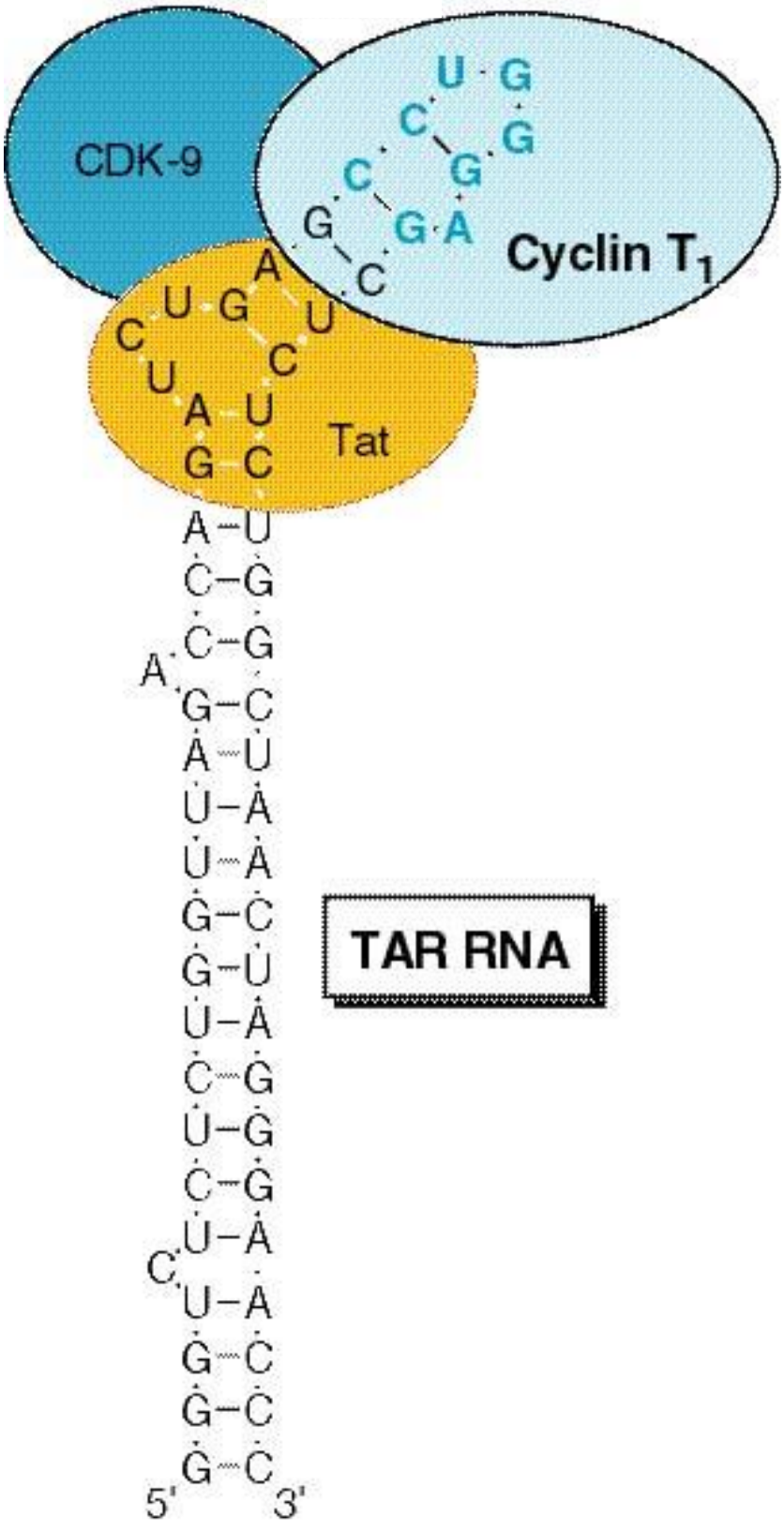
is composed of cyclin T1 and cyclin-dependent kinase 9 (Cdk 9) (155,165-167). Tat binds to the TAR structure on the viral RNA and recruits pTEFb complex through binding to cyclin T1 (155,155,165-168). Recruitment of pTEFb to TAR stimulates RNAPII phosphorylation by Cdk9 (169), and alters the substrate specificity of Cdk9 to include Ser5 phosphorylation of the CTD (170), resulting in the dissociation of DSIF and NELF (164). As a result, Tat facilitates transcription initiation. In addition, Tat also facilitates transcription elongation. Acetylation of Tat at Lys50 by p300 or hGCN5 dissociates cyclin T1 and Tat from TAR RNA (171-173) and transfers Tat to the elongating RNAPII complex where it recruits PCAF (p300/CREB binding protein-associated factor) via the PCAF bromodomain and enhances the transcriptional elongation of HIV-1 (174-177).

Third, Tat transactivtes HIV-1 transcription through the activation of NF-κB (178). Protein members of the Rel/NF-κB family bind to the enhancer element of the viral LTR (179,180). In the un-stimulated normal cell, NF-κB is retained in the cytoplasm by its inhibitor protein IκB-α (179). Tat promotes NF-κB activation through a change in the redox state of the cell and IκB-degradation (181-183).

1.5.2 Tat: Role in HIV pathogenesis

In addition to its crucial role in activating viral transcription, Tat is associated with a number of additional activities. Tat protein accumulates in the nucleus of HIV-infected cells, but can also act as a pleiotropic exogenous factor, because of its ability to induce various biological effects in different cell types (184). Extracellular Tat is actively released from HIV- infected cells (185,186) into the microenvironment and the circulation (187). Serum levels of HIV-Tat in HIV-

Figure 9. TAR RNA Structure. Tat recognition primarily requires interactions with the bulge region of TAR. In the presence of cyclin T1, conformational rearrangements in Tat permit interactions with the apical loop sequences (16). Figure adapted from (16). Figure was obtained with permission from Elsevier (Permission from the corresponding author as well as the publishing group are depicted in the appendix) (16).


CDK-9
Cyclin T1
Tat
TAR RNA
5'
3'

infected individuals vary between 0.1 - 1.0 ng/mL (187). Extracellular Tat targets different types of uninfected cells causing a variety of biological effects related to immune dysfunction.

Tat can affect many bystander cells though interaction with their receptors including CD26 (188), lipoprotein receptor-related protein (LRP) (189), CXCR4 (190,191) and hepran sulphate proteoglygans (HSPGs) (192). Tat protein is able to enter cells though endocytosis and can activate expression of various genes involved in inflammatory responses such as TNF-β, IL-6 and TGF-β (193,194). Tat is also known to repress cellular immune responses such as MHC-I presentation, IL-2, CD25 and anti-oxidative enzyme expression (193,195).

Immuno-suppression is one of the main characteristic features of AIDS, which develops in due course of time among HIV patients. Tat was demonstrated to play a role in immunosuppression and apoptosis of uninfected cells (196,197). Tat contributes to the immunosuppression of uninfected cells partly because of its enhancing effect on interferon -α (INF-α) production (197). INF-α has antiproliferative effects and at high levels is immunosuppressive. Both Tat and INF-α promote the generation of suppressor T cells in HIV-1 infected peripheral blood mononuclear cells (198). In addition, Tat directly inhibits antigen- and mitogen-induced lymphocyte proliferation (199,200). Tat also inhibits the natural immunity mediated by natural killer cells by blocking L-type calcium channels, which contributes to progressive immunosuppression during HIV-infection (201).

The downregulation of MHC I expression on HIV infected cells by HIV-Tat protein suggests another mechanism for the development of immune evasion and immune suppression (202). Since MHC I presents intracellular processed antigens to CD8+T cells, the downregulation of MHC I could severely affect CD8+T cell-associated immune responsiveness (195). Tat is

known to inhibit the production of nitric oxide (NO) by host macrophages (203). This in turn makes the host vulnerable to infections, because NO provides one of the first lines of defense against general opportunistic pathogens (203).

Next to immunosupression, Tat was shown to favour the spread of HIV-1 through different mechanism. First, Tat is able to increase, in dose dependent manner, the amount of HIV-1 correceptors, CCR5 and CXCR4, on the surface of CD4+ T cells (204). With more of these receptors on their surface these cells may be more susceptible to HIV-1 infection (204,205). Second, Tat was also shown to cause chemokine-like effects by promoting migration of several cell types, thereby recruiting cells to the vicinity of HIV-producing cells. Tat-induced migration has been reported for monocytes, endthoelial cells, B cells, dendritic cells and polymorphonuclear leucocytes (206-209).

1.5.3 Structure of HIV Tat protein

HIV-1 Tat is an 86-101 aa regulatory protein with a molecular weight of 12-14 kDa (185). Two forms of Tat are generated by translation from multiple spliced viral transcripts. One form is 72 amino acids in length and is encoded by a one-exon transcript (210). The other form is 101 amino acids in length and contains a C-terminal domain; this form is encoded by two exons separated by a non-coding region (193). Once transcription of full-length Tat mRNA is completed, the tat transcripts undergo double splicing, which results in removal of the non-coding region between the two coding exons (193). Residues 1-72 are encoded by the first exon and residues 73-101 are encoded by the second exon (Figure 10) (193,211). The difference in the position of translational stop codon in the second coding exon accounts for the range of size of two-exon Tat (86-101 amino acids). Malim *et al.* demonstrated that early in infection,

two-exon Tat is generated, while one-exon Tat appears after the onset of Rev expression (212).

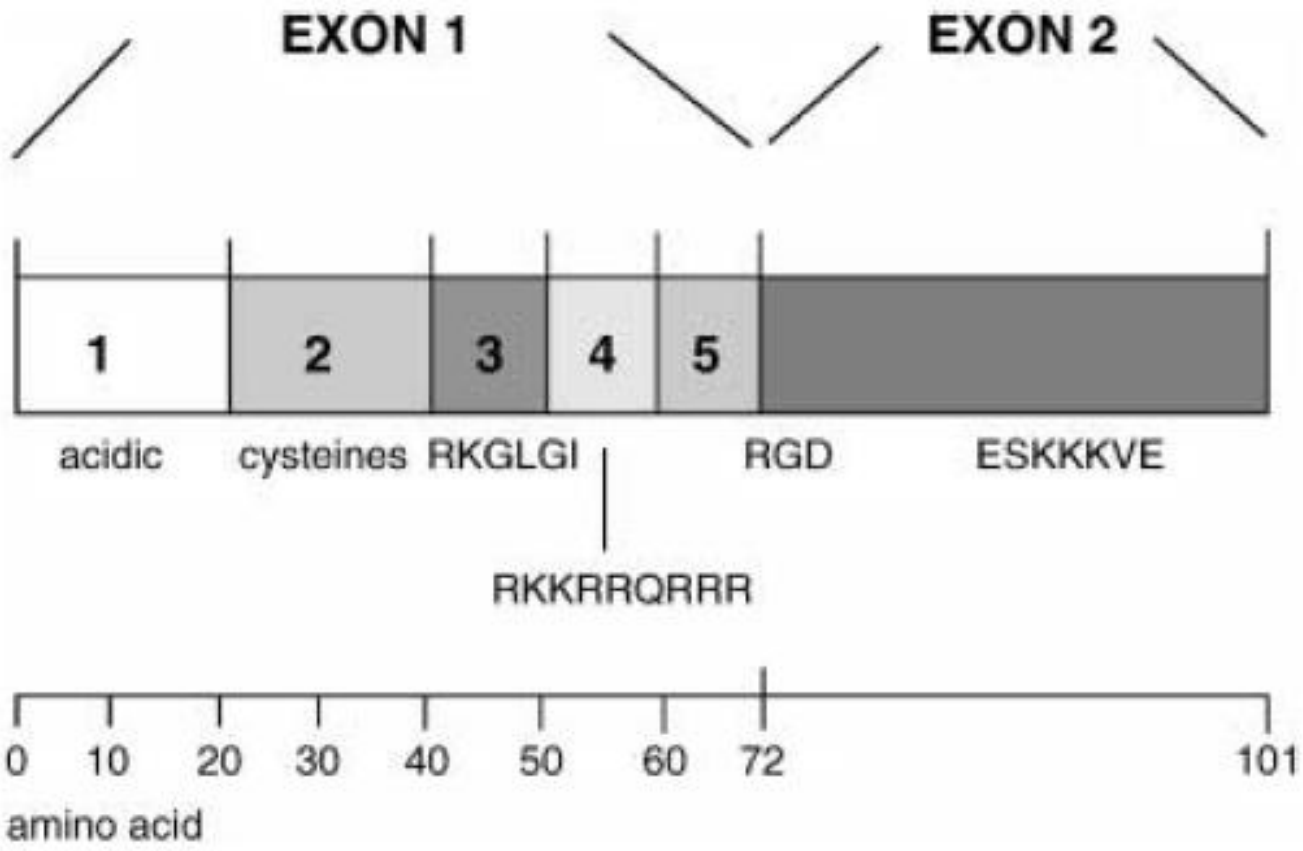
Tat protein is divided into six regions described in figure 10. Region I is rich in proline and includes residues 1 to 21 (Figure 10). Acidic amino acid residues present at positions 1 and 2 act as a pH sensor and play an important role in entry of Tat into cells. This region also has a conserved tryptophan at position 11 that is essential for release of Tat from infected cells (213) and its translocation into the cytosol of bystander cells (214).

Region II comprises residues 22 to 37 (Figure 10). This is a cysteine-rich region, having seven well conserved cysteines. Mutations in any of cysteines, except at position 31, result in loss of transactivation ability of Tat (215). Crystal structure of Tat-pTEFb complex shows that the conserved cysteines and histidine at position 33 are involved in Zn ion binding (216).

Region III ranges from residues 38 to 48 (Figure 10). This region has a conserved phenylalanine at position 38 and the conserved sequence ^{41}KGLGISYG^{48} at residues 41 - 48. The amino acid residues 41 to 45 are part of the minimal activation domain together with the cysteine-rich domain (217).Region IV consists of residues 49 to 59 (Figure 10). This region is rich in basic amino acid residues and has a well conserved sequence ^{49}RKKRRQRR^{56}. This sequence is essential for binding of Tat to TAR (217) and translocation of Tat to the nucleus (218). Residues 55 to 57 along with the acidic residues of the first region form a pH sensor, which controls the entry of Tat protein to the cytosol through the endosomal membrane (214). The basic domain is also required for efficient endocytosis of HIV-Tat protein (214).

Region V ranges from residue 60 to 72 (Figure 10). This region is a glutamine-rich region and involved in microtubule polymerization and Tat-mediated apoptosis of T-cells (219).

Figure 10: HIV-1 *Trans*-activator protein is composed of six regions. Linear representation of wild type Tat protein. Figure was obtained with permission from John Wiley and Sons (Permission from the corresponding author as well as the publishing group are depicted in the appendix) (194).


EXON 1
EXON 2
1
2
3
4
5
acidic
cysteines
RKGLGI
RGD
ESKKKVE
RKKRRQRRR
0
10
20
30
40
50
60
72
101
amino acid

Region VI, starts from residue 73 and can extend to either residues 88, 89 or 101 (coded for by the second exon). This region has a conserved ^{78}RGD^{80} motif and is involved in cell adhesion (220). An ^{86}ESKKKVE^{92} motif in this region is involved in NF-kB mediated transcription of HIV genes (221). Region VI is also responsible for modifications of cell cytoskeleton structure as well as T-cell signaling and activation (222).

1.5 CD127 and Tat

It has been established that active HIV replication is associated with marked decrease in the expression of CD127 on CD8 T-cells in HIV+ patients (82). Suppression of HIV replication with antiretroviral therapy was associated with an apparent recovery of CD127 on CD8 T cells (82). Our lab has recently shown that this down regulation of CD127 is mediated by the HIV Tat protein and results in poor CD8 T cell function, proliferation and perforin synthesis after stimulation with IL-7 (104,223,224). Recently, Tat's ability to bind to CD127 was shown to be mediated by Tat's N-terminus, where the removal of the N-terminus of Tat prevented Tat from co-immunoprecipitating with CD127 (225). Tat's ability to downregulate CD127 was shown to be time and dose sensitive and can be blocked with anti-Tat antibodies; also this downregulation can be reversed by removing Tat from the culture media (104,223,224).

Soluble Tat is secreted by infected CD4 T cells and taken up by neighbouring uninfected CD8 T cells through endocytosis. Once in the cytoplasm, Tat translocates to the inner leaflet of the cell membrane where it binds directly to the cytoplasmic tail of CD127 inducing receptor aggregation, internalization, and degradation by the proteasome (104,223,224). By removing CD127 from the cell surface, the HIV Tat protein is able to reduce IL-7 signalling and impair CD8 T-cell proliferation and function.

1.6 Hypothesis

In view of the pivotal role CD127 plays in CD8 T cells development, survival and function, and the established Tat induced downregulation of CD127; I hypothesize that HIV-1 Tat protein binds to a region on the CD127 cytoplamic tail.

1.7 Objectives

1) Construct a series of deletion mutants removing each of the four identified regions of the CD127 cytoplasmic domain
2) Transfect these clones into a cell line that doesn't express CD127.
3) Determine which region or regions of CD127 are required for Tat binding

2. Chapter 2. Materials and Methods

2.1 Reagents

2.1.1 Cytokines

Interleukin-2 (cat. no. PHC0027) was obtained from Gibco® by Life Technologies (Frederick, MD USA), and was resuspended in phosphate buffered saline (PBS) plus 0.1% bovine serum albumin (BSA) at a stock concentration of 10 ng/μl and stored at -80^{o} C.

2.1.2 Antibodies

2.1.2.1 Antibodies for Flow Cytometry

For flow cytometry anti-CD127-phycoerythrin (PE) (R34.34) fluorochrome-labeled monoclonal antibodies (cat. no. IM1980U) were purchased from Immunotech Beckman Coulter IO Test (Marseille, France). Fluorochrome labeled antibodies used for flow cytometry was titrated and used at saturating concentrations.

2.1.2.2 Antibodies for Western Blot and Co-Immunoprecipitation (CoIP) Assays

Polyclonal goat anti-human CD127 antibodies (cat. no. AF306PB) and monoclonal mouse anti-human CD127 antibodies (cat. no. MAB306) were obtained from R&D Systems (Minneapolis, MN). Monoclonal mouse anti-human β actin antibodies (C4) (cat. no. SC47778) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal anti- HIV-1 Tat antibodies (cat. no. 1102) were obtained from Immunodiagnostics (Woburn, MA). All primary antibodies in Western blots were used at a final concentration of 1 μg/ml. Secondary antibodies, donkey anti-goat (cat. no. HAF109) and goat anti-mouse (cat. no. HAF007) were purchased from R&D Systems (Minneapolis, MN).

2.2 Culture of Cell-lines

The human lymphoblastic CEM, Jurkat, A3, SupT, U9 and TALL 104 cell lines were obtained from the American Type Culture Collection (Manassas, VA). All T cell lines, except the TALL-104 cell line, were cultured at a density of 1 x 10^6 cells/ml in media comprised of RPMI 1640 (Gibco; cat. no. 11875-093; Grand Island, NY) supplemented with 100 U/ml penicillin (Sigma; cat. no. A0166; Saint Louis, MD), 100 µg/ml streptomycin (Sigma; cat. no. P0781; Saint Louis, MO), and 10% fetal bovine serum, FBS (Gibco; cat. no. 10082147; Carlsbad, CA). TALL-104 were cultured in ATCC-formulated Iscove's Modified Dulbecco's Medium (cat. no. ATCC 30-2005; Manassas, VA) supplemented with 100 U/ml penicillin (Sigma; Saint Louis, MD), 100 µg/ml streptomycin (Sigma; Saint Louis), 50 U/ml recombinant human IL-2 (Gibco; cat. no. PHC0026; Carlsbad, CA)., 2.5 µg /ml human albumin (Sigma; cat. no. A5843; Saint Louis, MO); 0.5 µg /ml D-mannitol (Sigma; cat. no. M9546; Saint Louis, MO) and 20% FBS. All cultures were maintained in a humidified incubator at 37^{o}C in the presence of 5% CO_2.

2.3 Primary Human CD8 T Cell Purification and Culture

Primary human CD8 T cells were isolated from blood of healthy volunteers with consent. Blood was drawn into syringes containing sodium heparin (20 units/ml), and peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Paque density centrifugation. In brief, 30 mL of blood was slowly layered above 12.5 mL of Ficoll-Paque^{TM} PLUS (GE Healthcare; Piscataway, NJ) and was centrifuged for 30 minutes at 16000 rpm. The PBMC fraction was transferred to fresh 50 mL tubes and PBMCs were pelleted and washed twice with PBS before resuspension in 800 µL of MACS buffer (PBS with 0.5% BSA and 2 mM EDTA) per 10^8 cells. CD8 MicroBeads

(Miltenyi Biotec, Auburn, CA, USA) were added to the cells in MACS buffer at a ratio of 1:4 per volume and the mixture was incubated on a rotator at 4°C for 25 minutes. The excess beads were then washed out by cell pelleting. After washing with PBS and resuspending the labeled cells in 2 mLs of magnetic-activated cell sorting (MACS) buffer, CD8 T cell purification was performed using the AutoMACS Isolation System (Miltenyi Biotech) using the cell sorter program 'possel_s' and CD8+ fractions were collected at the 'positive' fractions output. Purified CD8 T cells were cultured at a density of 1 x 10^6 cells/ml at 37^{o}C in media comprised of RPMI 1640 (Gibco; Grand Island, NY) supplemented with 100 U/ml penicillin (Sigma; Saint Louis, MD), 100 μg/ml streptomycin (Sigma; Saint Louis), MO and 20% FBS (Gibco; Carlsbad, CA). Cell purity was consistently >95% $CD8^+$ as assessed by flow cytometric analysis with only 2.6±0.9% $CD8^+CD3^-CD16^+CD56^+$ NK cells (111). All cultures were maintained in a humidified incubator at 37^{o}C in the presence of 5% CO_2. All research conducted using human blood was reviewed and approved by the Ottawa Hospital Research Ethics Board. The ethical consent forms are attached in the appendix.

2.4 SDS-PAGE Western Blotting

The human lymphoblastic CEM, Jurkat, A3 , SupT, U9 and TALL 104 cells(1 x 10^6 cells per condition) were collected by centrifugation at 6000 g for 10 minutes. Whole cell lysis was carried out in 50 μL RIPA buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 2 mM EDTA, 1% NP-40, 0.1% SDS plus 1X Halt Protease Inhibitor Cocktail (Roche Diagnostics, Mannheim, Germany) at 4^{o}C for 45 minutes. Protein concentration was quantified using the Bradford protein assay (Sigma; cat. no. B6916; Saint Louis, MO). Equal amounts of protein were added to 2x Laemmli

loading buffer (25 % glycerol, 125 mM Tris-HCl pH 6.8, 4% SDS, 10% β-mercaptoethanol, 0.01% bromophenol blue), boiled for 10 minutes and loaded onto an 8% or 12% SDS-polyacrylamide gel for detection of CD127 protein or Tat protein respectively. SDS-PAGE was carried out using the Mini-PROTEAN Tetra Cell apparatus (BioRad; Hercules, CA) at 120 volts. Proteins were then transferred to Immun-Blot PVDF membranes (Millipore, Billerica, MA, USA) using a semi-dry transfer apparatus (Bio Rad, Hercules, CA, USA) at 15 volts for 15 minutes. Membranes were then blocked with 5% nonfat dry milk in Tris-Buffered Saline plus 0.1% Tween-20 (TBST) for at least 1 hour. To detect CD127, membranes were incubated with a 1:1000 dilution of polyclonal goat anti-human CD127 antibody (R&D Systems) in 2% nonfat dry milk in TBST overnight at 4˚C with constant agitation. To detect Tat, membranes were incubated with a 1:1000 dilution of monoclonal anti-Tat (Immunodiagnostics) in 2% nonfat dry milk in TBST overnight at 4˚C with constant agitation. The next day, membranes were washed three times for 5 minutes each in TBST and then incubated with a 1:5000 dilution of horseradish peroxidase (HRP) conjugated to a secondary antibody in 2% nonfat dry milk in TBST for 1 hour at room temperature with constant agitation. For CD127 detection, a donkey anti-goat secondary antibody (R&D Systems) was utilized; while for the Tat detection, a goat anti-mouse secondary antibody (R&D Systems) was utilized. Membranes were again washed three times in TBST and proteins were visualized by chemiluminescence using the Western Blotting Luminol Reagent Kit (Santa Cruz Biotechology Inc. cat. no. SC2041) according to the manufacturer's instructions.

For re-probing loading controls, membranes were stripped for 30 minutes at 50^{o}C in stripping buffer (2% SDS, 62.5 mM Tris-HCl pH 6.8, 100 mM β-mercaptoethanol), washed 5 times with TBST, blocked with 5% nonfat milk in TBST for 1 h and then re-probed with a

monoclonal mouse anti-human β actin antibody followed by an HRP conjugated goat anti-mouse antibody (R&D Systems).

2.5 Cloning Plasmids

To examine the region required for HIV-1 Tat-CD127 binding, new constructs were generated using pCMV6-CD127 plasmid (OriGene, Rockville, USA) which encodes for the human IL7R-alpha (CD127) gene downstream of the CMV promoter (Figure 11). Deletions of each of the domains were carried out using the Stratagene Site Directed Mutagenesis Kit XL (Stratagene, La Jolla, CA, USA) which uses the ultra-high fidelity polymerase (Pfu-Ultra) for DNA amplification. To create the new clones with the deletions, the forward primers were designed to commence just downstream of the sequence to be deleted, while the reverse primers were designed to commence just upstream of the sequence to be deleted (Figure 12). All the primers were obtained from (Invitrogen; Grand Island, NY) and are summarized in (Table 2). One round of polymerase chain reaction (PCR) would amplify the coding sequence starting downstream of the sequence to be deleted all the way around to upstream of the sequence to be deleted (Figure 12). Hence, this design would produce a vector with the region/domain of interest deleted (Figure 12).

However, to introduce the specific point mutation, Try to Phe at position 449 (Y449F), the primers were designed with a mutation in the middle and were used as starting template to generate copies of the plasmid with the mutation (Figure 13). The sample reactions were prepared as follows: 50 ng of template DNA, 125 ng forward primer, 125 ng reverse primers, 5 µl of 10X reaction buffer, 1 µl of dNTP mix, 1 µl of Pfu ultra enzyme and completed to 50 µl with

Figure 11. pCMV6-CD127 plasmid back bone, containing the CD127 cDNA cloned downstream of the CMV promoter from OriGene®. (Permission from OriGene® is depicted in the appendix) (226).

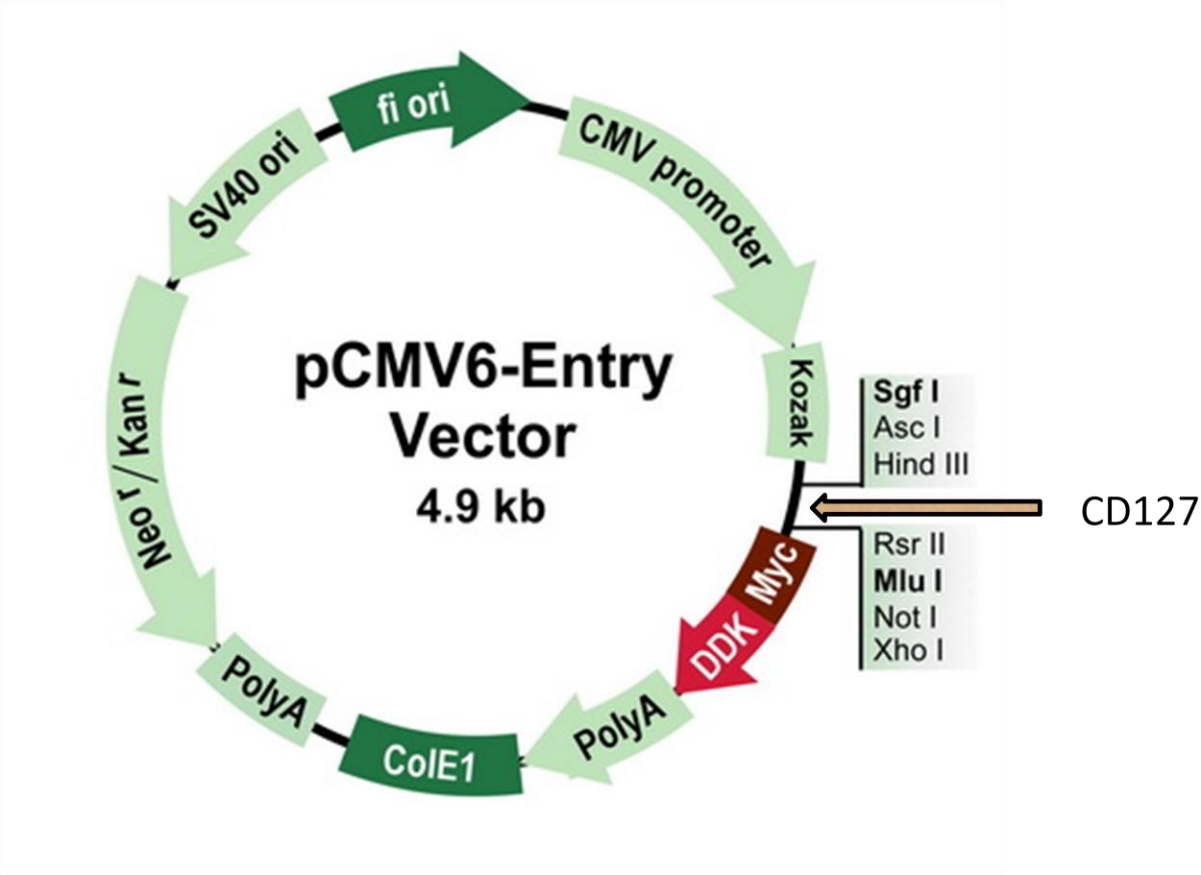

fi ori
CMV promoter
SV40 ori
Neo r / Kan r
pCMV6-Entry
Vector
4.9 kb
Kozak
Sgf I
Asc I
Hind III
CD127
Rsr II
Mlu I
Not I
Xho I
Myc
DDK
PolyA
PolyA
ColE1

Figure 12. Cloning strategy for pCMV-CD127 mutant plasmids. **1)** PCR was used to amplify the coding sequence regions just downstream all the way around to just upstream of the region to be deleted. **2)** The template was then digested by the addition of *Dpn I* enzyme. **3)**The PCR vector underwent a ligation process to generate plasmids lacking the deleted region. **4)** The mutant plasmid was then transformed into XL blue competent bacteria cells. **5)** DNA was then extracted from any colonies formed and sent to sequencing to confirm deleted region.

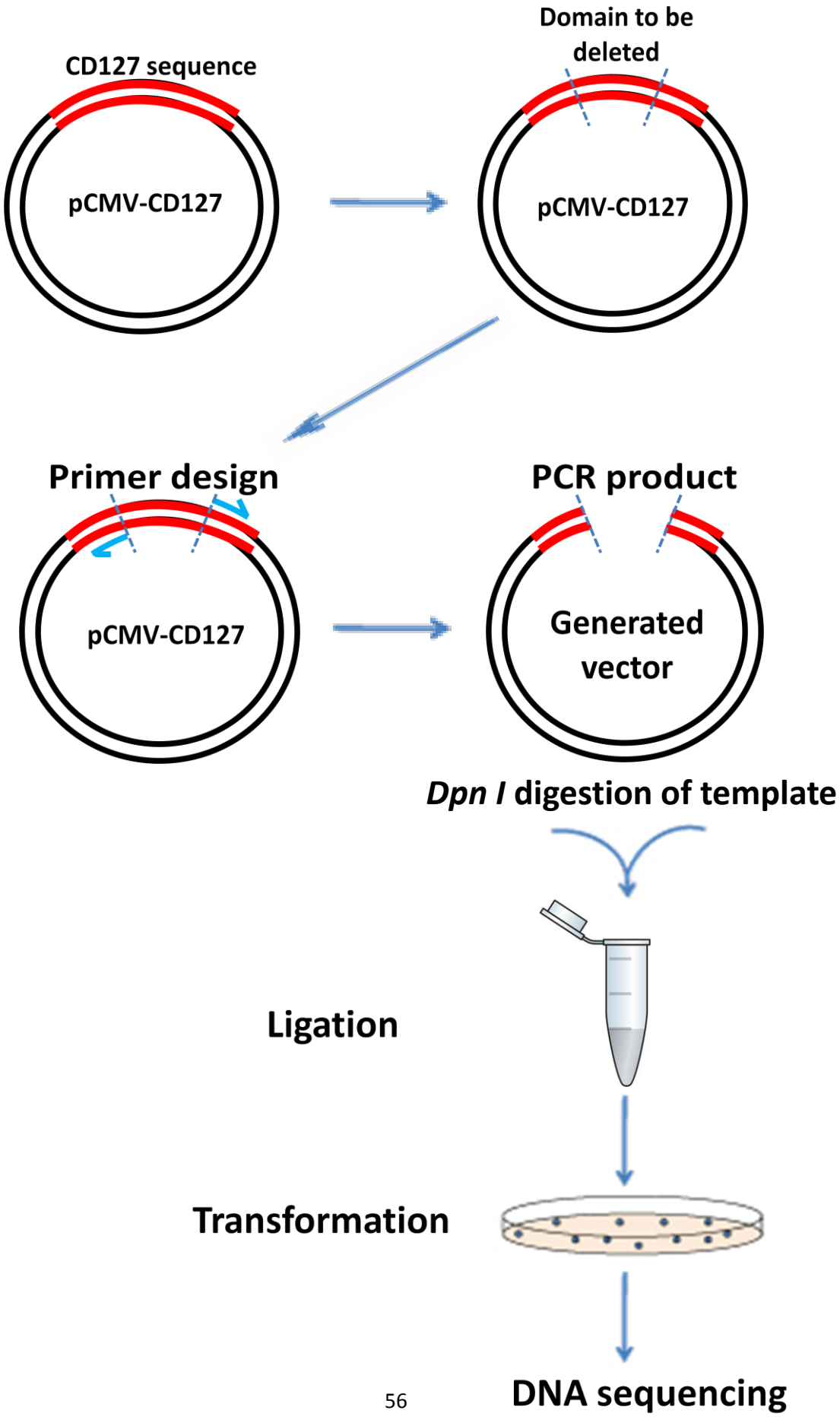
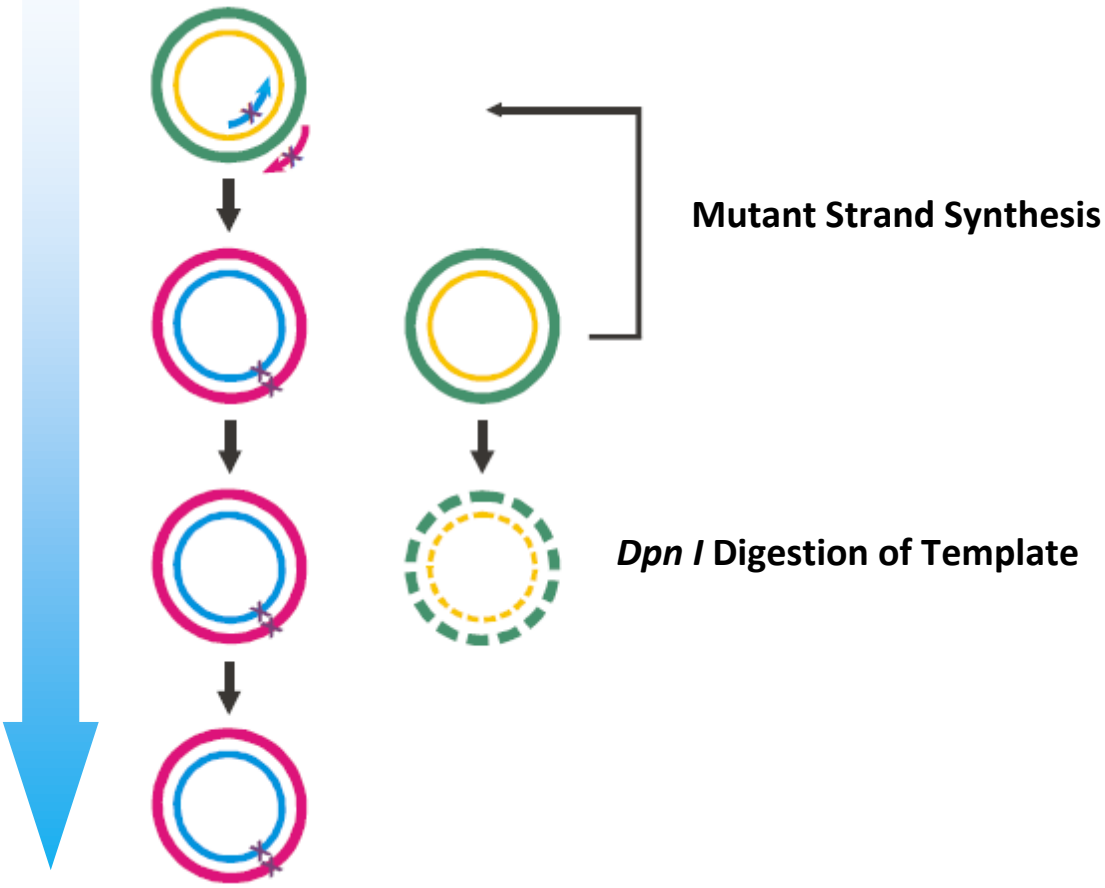

CD127 sequence
pCMV-CD127
Domain to be deleted
pCMV-CD127
Primer design
pCMV-CD127
PCR product
Generated vector
Dpn I digestion of template
Ligation
Transformation
DNA sequencing

Table 2. Primers used to clone the CD127 deletion mutants. The primers were designed using Oligonucleotide Properties Calculator. URL:
http://www.basic.northwestern.edu/biotools/OligoCalc.html.
The Y449F primers were designed using the Stratagene Site directed Mutagenesis primer designer program.

Primer Name	Mutation site w.r.t CD127 gene	Mutation achieved	Nucleotide sequence (5'-3')
Y449F-forward	1346-1348	Substitution Y449F	CAAATCAAGAAGAAGCAT**TC**GTCACCATGTCCAGCTTC
Y449F-reverse	1346-1348	Substitution Y449F	GAAGCTGGACATGGTGAC**GA**ATGCTTCTTCTTGATTTG
Δ acidic-forward	793-1071	Δ Acidic domain	CCAGAAAGCTTTGGAAGAGATTCAT
Δ acidic-reverse	793-1071	Δ Acidic domain	CCATAACACACAGGCCAAGAT
Δ box I-forward	813-840	Δ Box I domain	AAGACTCTGGAACATCTTTGTAAGAAA
Δ box I-reverse	813-840	Δ Box I domain	GATAGGCTTAATCCTTTTTTTCCATAAC
Δ serine-forward	1072-1185	Δ Serine domain	AATGGGCCTCATGTGTACCAG
Δ serine-reverse	1072-1185	Δ Serine domain	AGTGATGACTACATCCTCAGATG
Δ c-tail-forward	1186-1377	Δ C-tail domain	ACGCGTACGCGGCCG
Δ c-tail-reverse	1186-1377	Δ C-tail domain	CTTGCCACTCTCCCTGCA

Changes to original DNA sequence are in red: Y449F point mutation.

Figure 13. Overview of the QuickChange® II XL-site directed mutagenesis method. Figure was obtained with permission from Agilent Technologies © (Permission from Agilent Technologies © is depicted in the appendix) (227).


Mutant Strand Synthesis
Dpn I Digestion of Template

nuclease-free water. The PCR amplification conditions used were as follows: 95°C for 2 min for initial enzyme activation, then 30 cycles of 95°C for 30s, 53°C for 1 min, then 72°C for 12 min 36 sec. Since the non-mutant plasmid template was originally generated in *E. coli*, it is methylated. Therefore, to get rid of the non-mutant DNA template, the reaction mix was incubated with *DpnI* to eliminate methylated DNA (Figure 12 and 13). The digestion took place overnight at 37 ^{0}C. Ligation of the vector was the final step in clone generation (Figure 12). The ligation conditions used were as follows: 51 µl PCR-amplified product, 3 µl of T4 ligase (New England BioLabs; Ipswich, MA) and 6 µl of 10x ligation buffer (New England BioLabs; Ipswich, MA). Ligation reactions were carried out overnight at 15^{o}C.

2.6 Plasmid isolation and production

After molecular manipulations were carried out, plasmids were transformed into XL1-Blue *E. coli* cells using a standard heat shock method: 50 µL of competent XL1-Blue (Stratagene, La Jolla, CA, USA) were added to 10 µL of cloned construct on ice and incubated for 15 minutes. Cells underwent heat shock at 42^{o}C for 30 seconds, and then were immediately placed on ice for 2 minutes. This was followed by the addition of 250 µl of super optimal broth with catabolic repressor 100 µg/mL (SOC) media (Sigma; cat. no. S1797; Saint Louis, MO). Transformed cells were shaken at 37^{o}C for 1 hour. Next, 200 µL of cells were then plated onto a 9 cm agar plate containing kanamycin (kan; Sigma; cat. no. 60615**;** Saint Louis, MO) at 50 µg/mL concentration (Figure 12). The following day, colonies were picked and inoculated into 2 mL of Luria broth (LB; Sigma; cat. no. L3522**;** Saint Louis, MO) with kan at 50 µg/mL, and placed at 37^{o}C overnight with

shaking. The following day, mutant plasmids were isolated using the plasmid DNA purification kit NucleoSpin® Plasmid QuickPure kit (miniprep) or the NucleoBond® Xtra Maxi kit (maxiprep) both from Macherey-Nagel (Düren, Germany).

2.7 Plasmid Identification and sequencing

2.7.1 Agarose gel electrophoresis

To identify the plasmids with deletions, the plasmid was digested to reveal the CD127 cDNA apart from the vector backbone. Preliminary results helped to initially identify deletions which were then confirmed by DNA sequencing (Figure 12). The digestion conditions were as follows: 20 µl plasmid (from the 50 µl miniprep), 1 µl AsiSI, 1 µl Mlu I, 2.5 µl 10x NEB buffer 4 (New England BioLabs; Ipswich, MA) and 100 µg/mL BSA in a final volume of 25 µL. Digestion took place overnight at 37^{o}C. The following day, the digestion was terminated by incubating the samples at 85^{o}C for 20 minutes. Following digestion termination, 6x loading buffer (30% glycerol, 0.25% bromophenol blue and 0.25% xylene cyanol FF) was added to the digested plasmids and the DNA was separated on a 1% agarose gel run at 80 volts for 1 hour. DNA bands were visualized under ultraviolet light using ethidium bromide.

2.7.2 Sequencing

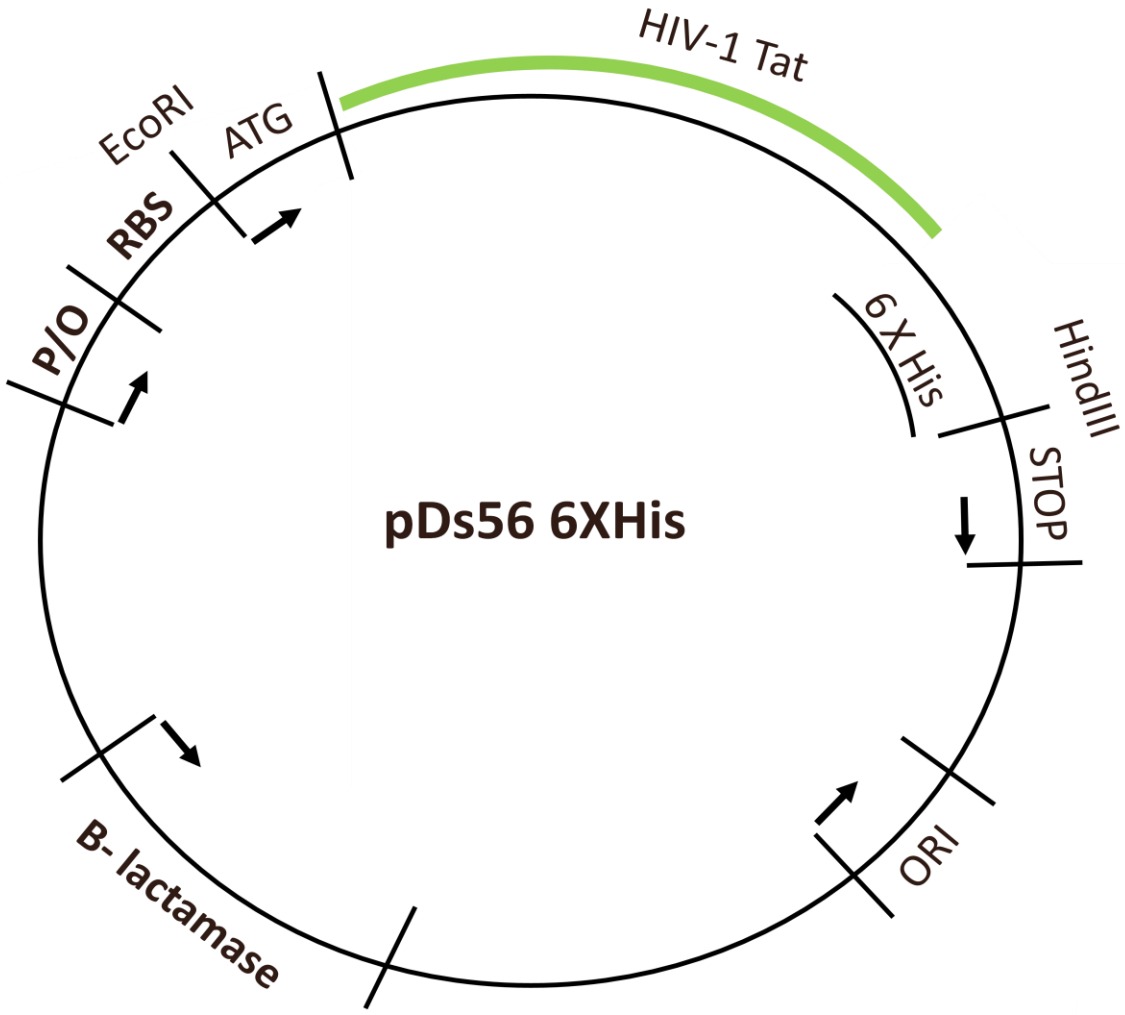
All sequences were verified by Sanger sequence analysis at the StemCore facility at the Ottawa Hospital Research Institute (OHRI).

2.8 Tat production

Recombinant Tat protein was produced in the XL2 Blue strain of *E. coli* and isolated over Ni-TED columns. XL2 Blue *E. coli* were transformed with pTatC6H-1 plasmid which was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH from Dr. Abhay Patki and Dr. Michael Lederman. This vector has a pDS56 plasmid backbone and encodes for the B clade Tat variant BH10 linked to six histidine residues at its C-terminal end (Figure 14).

Bacteria were grown in 500 mL of Luria Broth (LB) with 100 µg/mL of ampicillin at 37^{o}C with agitation to an OD_{600} of 0.2-0.4 (approximately 12-14 hours) before Tat production was induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for 3 hours. Bacteria were pelleted and resuspended in 3 mL of sonication buffer (50 mM NaH_2PO_4, 300 mM NaCl and 10 mM β-mercaptoethanol, pH 8.0) supplemented with 2 mg/mL of chicken egg white lysozyme (Sigma-Aldrich; cat. no. L3790; Louis, USA) and 10 mM β-mercaptoethanol (Sigma-Aldrich, Louis, USA) and incubated on ice for 1 hour to allow digestion of bacterial cell walls. Bacterial lysates were then sonicated on ice for 10 seconds (each at AMP=30%) over 3 minutes pausing 10 seconds in between pulses (Vibra-Cell™, Sonics & Materials Inc., Newtown, CT, USA). Lysates were then centrifuged at 10 000 g for 15 minutes at 4^{o}C. The soluble fraction was then passed through a Ni-TED Superflow column (Macherey-Nagel, Duren, Germany) and the column was washed with five column volumes of wash buffer (50 mM NaH_2PO_4, 300 mM NaCl, and 10 mM β-mercaptoethanol, pH 8.0). Histidine tagged Tat protein (Tat6xHis) was eluted using 1 mL of

Figure 14: The pTatC6H-1 plasmid containing Tat protein on a pDS56 vector backbone.


HIV-1 Tat
EcoRI
ATG
RBS
P/O
6X His
HindIII
STOP
pDs56 6XHis
B- lactamase
ORI

elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole and 10 mM β-mercaptoethanol, pH 8.0). Proteins were separated into 500 µL aliquots in 1.5 mL tubes and frozen slowly using an isopropanol jacket and stored at -80^{o}C.

The identity of the purified Tat-1 protein was confirmed by Western blot analysis as described above. PVDF membranes were probed for Tat using monoclonal anti-Tat antibodies (Santa Cruz, CA). Goat anti-mouse-horseradish peroxidase (HRP) (R&D systems; Minneapolis, MN) was used as a secondary antibody. Protein bands on Western blots were detected using the Western Blotting Luminol Reagent Kit (Santa Cruz Biotechology) as per the manufacturer's instructions. Protein purity was confirmed by silver stain analysis as described in 2.9.

Recombinant Tat protein was quantified via densitometry of silver stained polyacrylamide gels using commercially purchased Tat as a standard (Advanced BioScience Laboratories Inc.; Rockville, MD).

2.9 Polyacrylamide Gel Silver Staining

Tat6xHis protein was separated on a 12% polyacrylamide gel and placed in fixing buffer (40% ethanol, 10% acetic acid, and 50% ddH_2O) for 30 minutes with constant agitation at room temperature. The gel was then washed once for 10 minutes in rinse buffer (50% ethanol and 50% ddH_2O), and then twice in ddH_20 for 10 minutes. Next, the gel was sensitized in 0.02% sodium thiosulfate for 1 minute; then washed twice for 5 minutes in ddH_20. To detect protein, the gel was then placed in cold staining solution (0.1% silver nitrate) for 30 minutes with

constant agitation before being developed in 3% sodium carbonate and 0.03% formaldehyde. The developing reaction was quenched with 1% acetic acid.

2.10 Flow cytometry

Surface CD127 protein expression was analyzed by incubating 100 µL of cells ($1x10^5$ cells) with 4 µL of anti-CD127-PE antibody for 20 minutes to allow for antibody binding in the dark at room temperature.

Cells were then analyzed by a Cytomics FC500 flow cytometer (Beckman Coulter; Indianapolis, IN). Live cells were gated on the basis of side and forward scatter and at least 10,000 events were recorded for each sample. Auto-florescence from unstained cells was used to set the cut-off gate for $CD127^+$ staining. Resulting profiles were analyzed with FCS Express 2 software (De Novo, Los Angeles, CA, USA).

2.11 Cell Transfection

Transfection of Jurkat T cells with wt CD127 plasmid and CD127 mutant plasmids

In brief, plasmid DNA used for transfection was isolated from XL1 Blue competent *E. Coli* cells using the NucleoSpin® Plasmid QuickPure kit (miniprep) or the NucleoBond® Xtra Maxi kit (maxiprep) both from Macherey-Nagel. Jurkat cells were seeded at a concentration of 0.9 million cells/ml and were cultured overnight in RPMI-1640 media. The next day, 10 µg plasmid DNA as well as Jurkat cells were suspended in 100 µL of Nucleofector® solution at $2.5x\ 10^6$ cells per condition and cell transfection was achieved using the Nucleofector II device with the X-005 program setup. Post-nucleofection, cells were allowed to recover in pre-warmed serum-free

RPMI-1640 at a concentration of 2.5 x10^6 cells/ml for 4 hours before being transferred into RPMI plus 10% FCS at a concentration of 1x10^6 cells/ml.

2.12 Immunoprecipitation

IP of CD127 from Transfected Jurkat Cells

All immunoprecipitation (IP) buffers were supplemented with 1X Halt Protease Inhibitor Cocktail (Roche Diagnostics)**.** Twenty-four hours after transfection, five million Jurkat cells were centrifuged at 6000 g for 10 minutes and lysed in 100 µl of Lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.5% NP-40, 10% glycerol, 2 mM DTT) on ice for 1 hour. After centrifugation at 11 000 g for 10 minutes to pellet cellular debris, supernatants were diluted with 450 µl of Dilution buffer (10% glycerol, 20 mM Tris-HCl pH 8.0, 0.5 mM EDTA, 1 mM DTT, 1 mM PMSF, and 1 mg/ml BSA) and were pre-cleared to minimize background by incubating with 50 µL of Protein G Agarose beads (cat. no 20398; Pierce-Thermo Fisher Scientific, Waltham, MA, USA) on a rotary shaker for 1 hour at 4^{o}C. Protein G beads were removed by centrifugation at 17 000 g. Pre-cleared lysates were then incubated with 2.5 µg of polyclonal goat anti-human CD127 antibody or isotype control goat antibody (R&D Systems) and 50µl of Protein G agarose beads on a rotary shaker for 6 hours at 4^{o}C. Antibody–protein G complexes were pelleted at 17 000 g for 2 minutes and washed four times with wash buffer (10 % glycerol, 100 mM NaCl, 20 mM Tris-HCl pH 8.0, 0.5 mM EDTA, 1 mM DTT, 1 mM PMSF, 0.1 % NP-40) for 2 minutes each time at 4^{o}C. Proteins were then eluted off the beads by adding 60 µL of 3X Laemmli loading buffer, boiled for 10 minutes at 100^{o}C, and loaded onto an 8% SDS polyacrylamide gel. Immune

complexes were separated by SDS-PAGE and probed for the presence of CD127 protein by Western blot. SDS-PAGE and transfer were carried out as described in 2.4.

2.13 Co-Immunoprecipitation

Co-IP of CD127 and Tat from Transfected Jurkat Cells

All Co-IP buffers were supplemented with 1X Halt Protease Inhibitor Cocktail (Roche Diagnostics). Five million transfected Jurkat cells were centrifuged at 6000 g for 10 minutes and lysed in 100 µl of Lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.5% NP-40, 10% glycerol, 2 mM DTT) on ice for 1 hour. After centrifugation at 11 000 g for 10 minutes to pellet cellular debris, supernatants were diluted with 450 µl of Dilution buffer (10% glycerol, 20 mM Tris-HCl pH 8.0, 0.5 mM EDTA, 1 mM DTT, 1 mM PMSF, and 1 mg/ml BSA) and were pre-cleared to minimize background by incubating with 50 µL of Protein G Agarose beads (Pierce-Thermo Fisher Scientific) on a rotary shaker for 1 hour at 4^{o}C. Protein G beads were removed by centrifugation at 17 000 g. To determine the minimum amount of Tat to utilize, the pre-cleared supernatants were incubated with 1 µg, 5 µg or 10 µg Tat6xHis protein for 1 hour at 4^{o}C. For all other Co-IP experiments, 10 µg Tat6xHis protein was used. CD127 was then immunoprecipitated by incubating the supernatants with 2.5 µg of polyclonal goat anti-human CD127 antibody or isotype control goat antibody (R&D Systems) and 50µl of Protein G agarose beads on a rotary shaker for 6 hours at 4^{o}C. Antibody–protein G complexes were pelleted at 17 000 g for 2 minutes and washed four times with wash buffer (10 % glycerol, 100 mM NaCl, 20 mM Tris-HCl pH 8.0, 0.5 mM EDTA, 1 mM DTT, 1 mM PMSF, 0.1 % NP-40) for 2 minutes each time at 4^{o}C. Proteins were then eluted off the beads by adding 60 µL of 3X Laemmli loading

buffer, boiled for 10 minutes at 100^{o}C, and loaded onto a 12% SDS polyacrylamide gel. Immune complexes were separated by SDS-PAGE and probed for the presence of Tat protein by Western blot. SDS-PAGE and transfer were carried out as described in 2.4.

3. Chapter 3: Results

3.1 Rational

Impaired cell mediated immunity is the clinical hallmark of HIV infection yet the manner in which CD8 T-cells are disabled is not yet understood. IL-7 signalling is essential for normal CD8 T-cell development and function. Our lab has previously shown decreased expression of the IL-7 receptor alpha-chain (CD127) on circulating CD8 T-cells in HIV+ patients (82). Furthermore, suppression of HIV replication with antiretroviral therapy was associated with an apparent recovery of CD127 on CD8 T cells (82). Although the exact mechanism by which CD127 is down regulated in vivo has not been definitely established, our lab has shown soluble HIV Tat protein when added to CD8 T-cells in culture is able to specifically down regulated CD127 at the cell surface (223). The Tat mediated downregulation of CD127 was shown to be time and dose dependent, specific for CD127, can be blocked using anti-Tat antibodies, and can be reversed by removing Tat from the culture media (223). Furthermore, the decreased CD127 expression was shown to result in impaired CD8 T-cell proliferation and perforin synthesis after stimulation with IL-7.

Tat protein is secreted by infected CD4 T cells and is taken up by neighbouring uninfected CD8 T cells though endocytosis. Once in the cytoplasm, Tat translocates to the inner leaflet of the cell membrane where we believe, it binds to the cytoplasmic tail of CD127. Our lab has previously shown that purified Tat protein physically interacts with CD127 on CD8 T cells *in vitro* (223). More specifically, our lab has shown that Tat interacts with the cytoplasmic domain of CD127 through its N-terminal domain (225). By binding to CD127, Tat then mediates the accelerated internalization and proteasomal degradation of CD127. By removing CD127

from the cell surface, the HIV Tat protein is able to reduce IL-7 signaling and impair CD8 T-cell proliferation and function. Therefore, to better understand the molecular details of CD127/Tat interactions, I have set out to determine the CD127 region(s) that bind to Tat. This work can have great implications leading to novel drug targets which may disrupt the CD127/Tat interaction and possibly result in improved IL-7 signalling and improved cell mediated immunity.

In order to characterize the CD127/Tat physical interaction, I constructed a series of CD127 deletions to generate mutant proteins each lacking a successive domain of the wild type CD127 cytoplamic tail. The 195 amino acid CD127 cytoplasmic tail has been divided into four domains: a membrane-proximal box I domain, an acidic domain, a serine-rich domain followed by a C-terminal tail which contains three tyrosine residues (118). Once each domain is successively removed, further experimentation can be performed to characterize the CD127/Tat interaction.

Tat is known to affect the phosphorylation, ubiquitination, methylation and/or acetylation states of numerous host proteins (228). Therefore, we wanted to investigate whether Tat induces post-translational modifications in CD127. The binding of IL-7 to its receptor results in phosphorylation of Y449 by Jak1 (95) or Jak3 (135) and subsequent CD127 downregulation. Although initial removal of the receptor from the cell membrane can occur in the absence of Y449 phosphorylation, Y449 phosphorylation is required for subsequent CD127 degradation by the proteasome (225). Since Tat is known to interact with several cellular kinases (229,230), the potential for Tat to induce CD127 phosphorylation in a manner similar to IL-7 was investigated by generating a plasmid with a point mutation at Y449.

In this chapter, I describe a method to produce mutant plasmids and their expression in a cell line. The mutant plasmids sequences were confirmed and their protein expression was confirmed by Western blot and flow cytometry. I also describe a method to produce pure wild type Tat protein. The identity of Tat protein was confirmed by Western blot.Finally, I use the CD127 mutant protein and the Tat produced to further elucidate the mechanisms of CD127/ Tat interaction.

3.2 CD127 Region Required for Tat binding

3.2.1 Production and Expression of CD127 Mutant Proteins in a Cell Line

3.2.1.2 Production of CD127 mutant clones

The aim was to elucidate the region within CD127 that binds to Tat. To accomplish this, I used the pCMV6-CD127 plasmid containing the CD127 cDNA cloned downstream of the CMV promoter (OriGene). The pCMV6-CD127 plasmid was utilized to construct a series of deletion mutant proteins removing each of the identified regions within the CD127 cytoplamic domain (Figure 7). We reasoned that the expression of these mutants in a cell line lacking CD127 would allow us to determine the region required for Tat binding. Plasmids encoding CD127 lacking the box1 domain [Δ Box1; Δ 9 amino acid (aa); Δ 27 base pairs (bp)], acidic domain (Δ Acidic; Δ 93 aa; Δ 279 bp), the serine domain (Δ Serine; Δ 38 aa; Δ 76 bp) and the c-tail (Δ C-tail; Δ 64 aa; Δ 192 bp) along with the plasmid containing a point mutation Y449F were successfully created and purified as described in Chapter 2. A summary of all the plasmids harboring mutations of the CD127 cytoplasmic tail are depicted in figure 15. The clones were digested and ran on an agarose gel revealing the CD127 cDNA as seen in figure 16. As expected, CD127 cDNA of

Figure 15: Linear representations of CD127 mutants. Standard molecular techniques were used to generate the series of CD127 mutant plasmids shown.

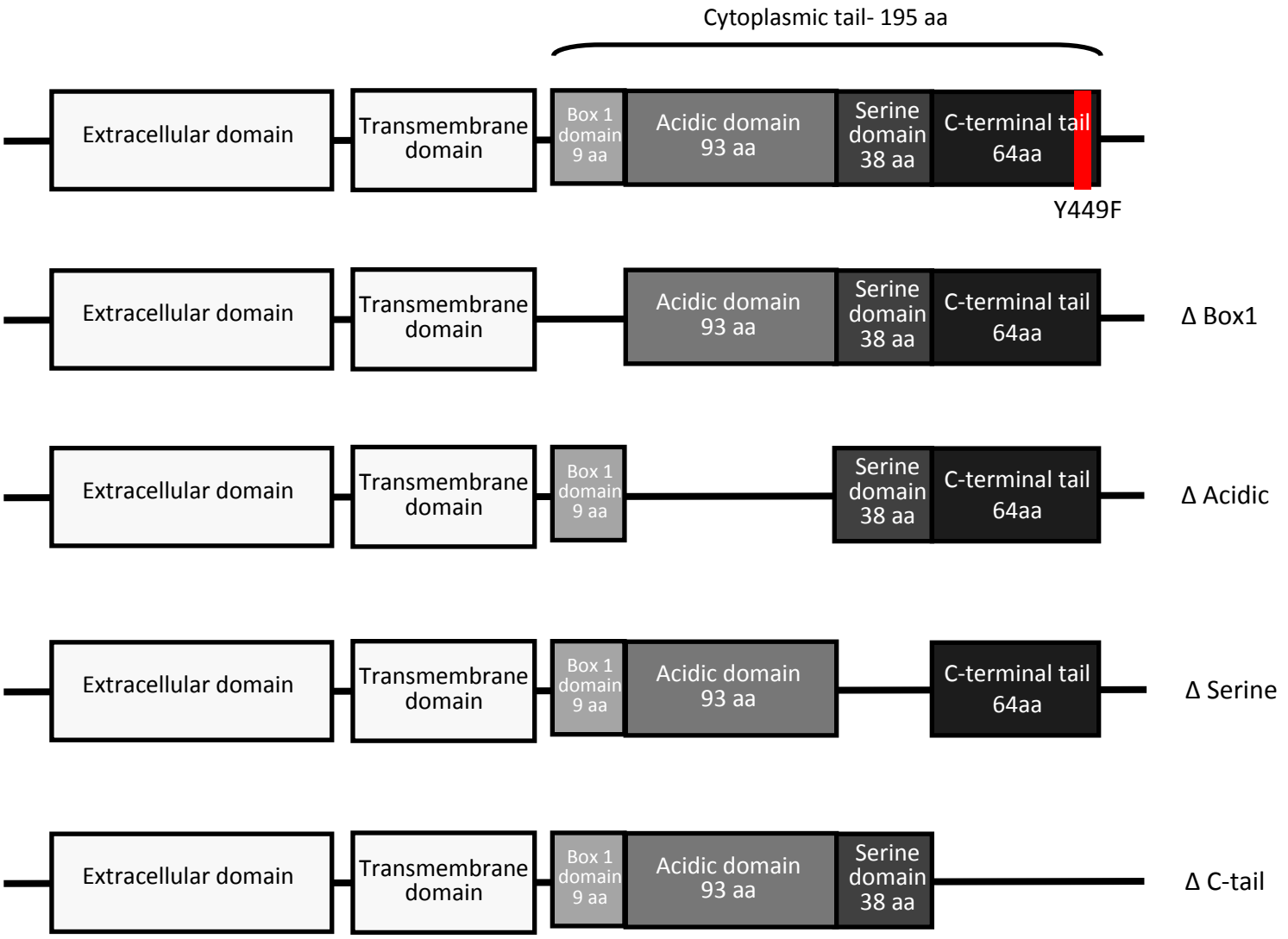
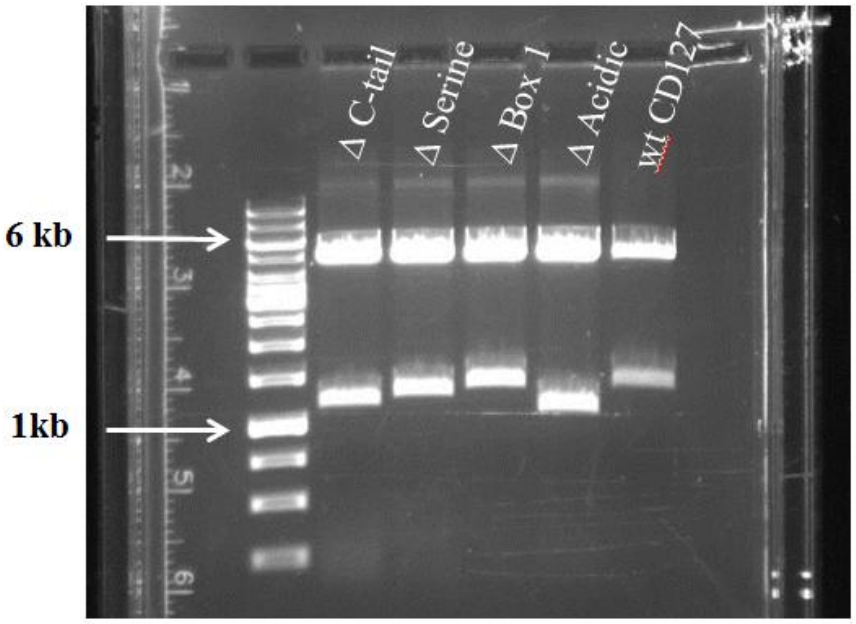

Cytoplasmic tail- 195 aa
Extracellular domain
Transmembrane domain
Box 1 domain 9 aa
Acidic domain 93 aa
Serine domain 38 aa
C-terminal tail 64aa
Y449F
Δ Box1
Δ Acidic
Δ Serine
Δ C-tail

Figure 16: Agarose gel showing wild type and mutant CD127 cDNA. Plasmid DNA was digested with MluI and FseI.


6 kb
1kb
Δ C-tail
Δ Serine
Δ Box 1
Δ Acidic
wt CD127

mutants with deletions ran further than wild type CD127 cDNA due to the smaller size. The Δ acidic clone, with a 279 bp deletion, ran further compared to the full length CD127 cDNA followed by Δ c-tail clone, 192 bp deleted, then Δ serine clone, 76 bp deleted, and finally Δ box1 clone, 27 bp deleted. To confirm the cDNA sequence, each clone was verified by Sanger sequence analysis at the StemCore facility at the Ottawa Hospital Research Institute (OHRI).

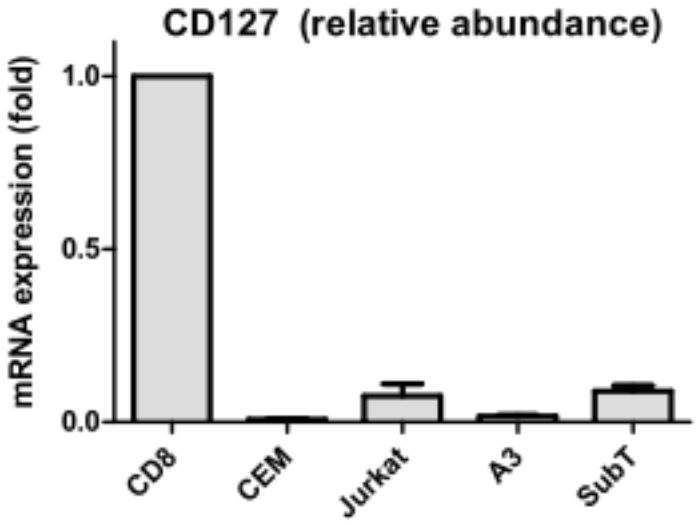
3.2.1.2 Selection of a T cell lacking CD127

Since we wanted to express wild type and mutant CD127 in a eukaryotic T cell line, we investigated the CD127 expression in five potential cell lines. A cell line lacking endogenous CD127 serves as the best option for assessing the effects of Tat on CD127 mutants post transfection. The CD127 mRNA and protein expression was determined in the following T cell lines: CEM, Jurkat, A3, SupT and TALL-104. Expression of CD127 mRNA was determined by qPCR. Jurkats and SupT showed low level CD127 mRNA expression whereas CEM, A3 and TALL-104 CD127 mRNA levels were negligible (Figure 17a and 18a). Interestingly this was not reflected at the protein level, all cell lines showed a negligible level of CD127 protein as determined by Western blot (Figure 17b and 18b). All the cell lines with the exception of TALL-104 originate from a CD4 T cell linage, while TALL-104 originates from a CD8 T cell linage. We wanted to continue the experimentations in the TALL-104 cell line since our model is based on CD8 T cells and not CD 4 T cells. However the TALL-104 T cell line quickly proved to be a difficult cell line to culture. The growth was only optimal at 50 U/ml recombinant human IL-2 and 2.5 µg /ml human albumin, even still, the doubling capacity was slower than that of the other T cell lines. The TALL-104 cell line had a doubling time of 10 – 12 days while the other T cell lines had

Figure 17: (a) PCR data and (b) western blot analysis showing the expression of CD127 in CD8 T cell, CEM, Jurkat, A3 and SupT cell lines. The PCR data was generated by Dr. Al Ghazawi.

A.

mRNA transcripts



N=3

B.

Protein

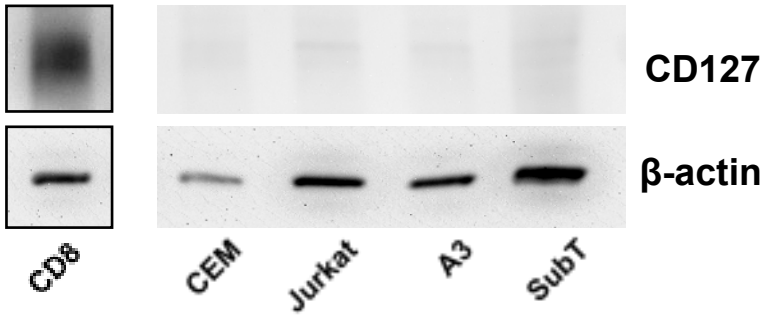
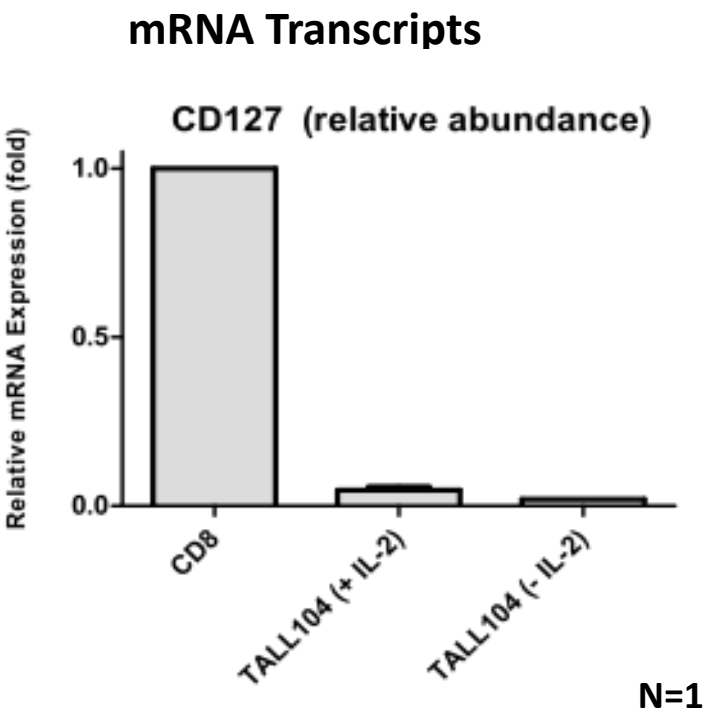
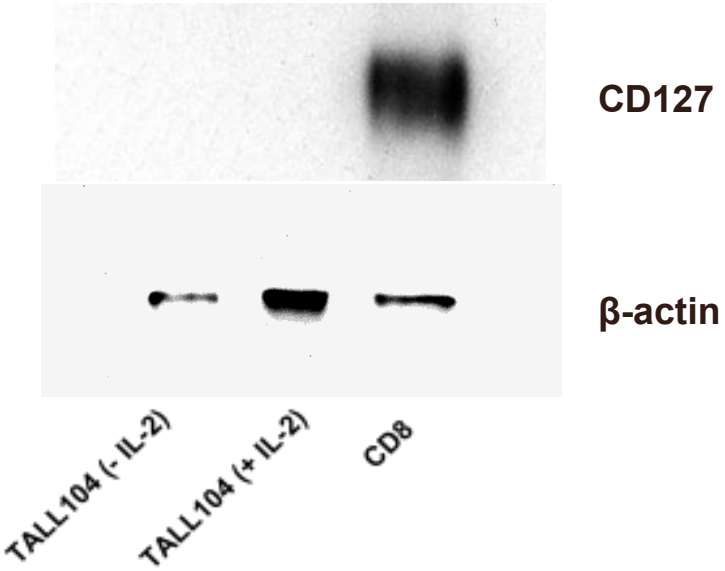


Figure 18: (a) PCR data and (b) western blot analysis showing the expression of CD127 in CD8 T cell and TALL-104. The PCR data was generated by Dr. Al Ghazawi.

A.


mRNA Transcripts
CD127 (relative abundance)
Relative mRNA Expression (fold)
1.0
0.5
0.0
CD8
TALL104 (+ IL-2)
TALL104 (- IL-2)
N=1

B.


Protein
CD127
β-actin
TALL104 (- IL-2)
TALL104 (+ IL-2)
CD8

a doubling time of 4 – 5 days. The Jurkat cell line has precedence due to expression of a Jurkat-CD127 cell line by Dr. van Lier's group (231). Therefore we proceeded with the Jurkat cell line.

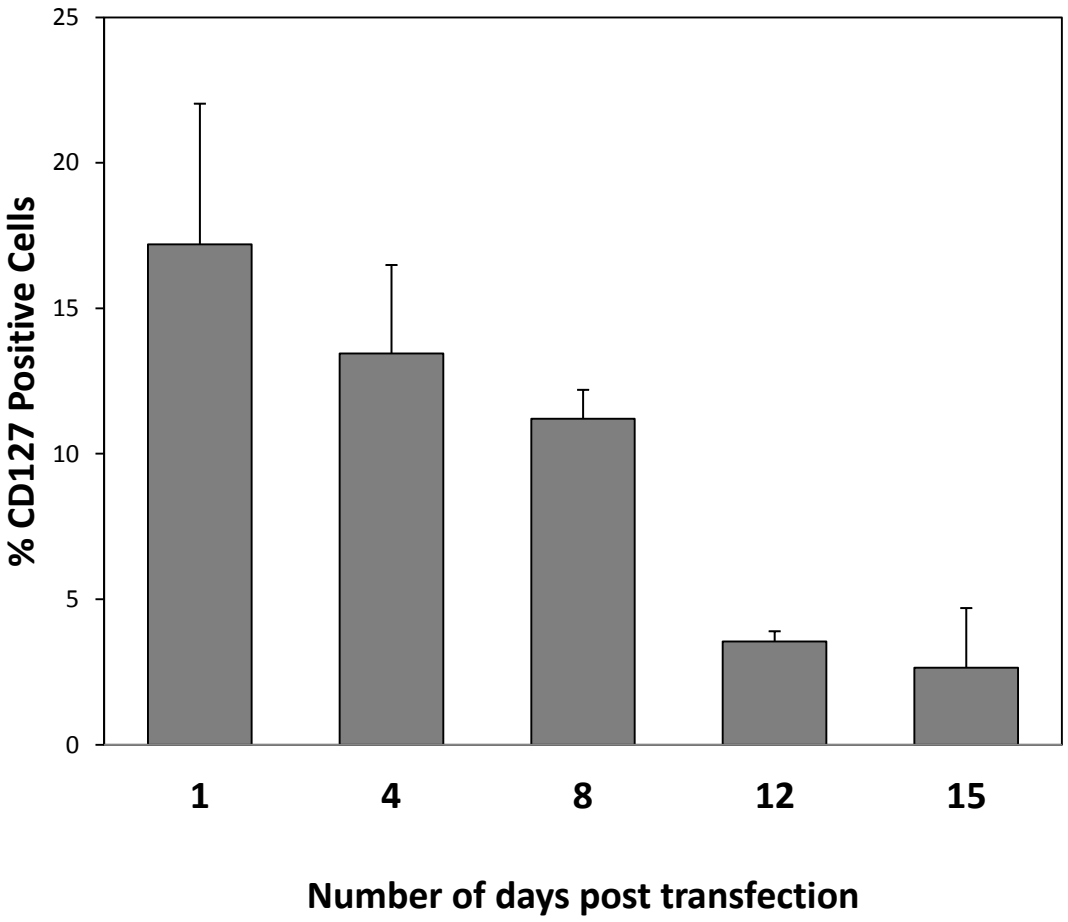
3.2.1.3 Expression of mutant clones in a T cell line

We next wanted to confirm that the plasmids I cloned are capable of expressing CD127 within a human T cell line. The transfection of suspension cells, such as Jurkat cells, is known to be inherently difficult; therefore we utilized a nuclefection system which has a specific protocol for the transfection of suspension cells. This system greatly increased transfection efficiency when compared to *Trans*IT®-Jurkat Transfection reagent and PromoFectin Transfection reagent (data not shown). The wild type CD127 plasmid was utilized to optimize the transfection conditions before continuing with the mutant plasmids.

Since 24 hours post transfections showed the highest level of surface CD127 expression, the rest of the experiments were carried out at this time point, see figure 19. The full length and deletion mutants were transfected using the nucleofection system at the optimized conditions as described in Chapter 2. CD127 protein expression on successfully transfected cells was measured by flow cytometry at 24 hours. As shown in figure 20, CD127 was effectively expressed on the cell surface from the plasmids transfected into Jurkat cells. CD127-positive cells in the different mutant transfections varied between 15% - 30% over three experiments. With the wild-type construct, 18 ± 4% of cells expressed CD127. The Y449F clone had the lowest CD127-positive cells at approximately 5 ± 1% while Δ box1 clone had the highest at 30 ± 4%

To confirm the flow cytometry data, we also sought to detect CD127 protein expression by western blotting. This turned out to be a challenge likely because of low protein expression in transfected cells and lower transfection efficiencies. In an attempt to increase the sensitivity,

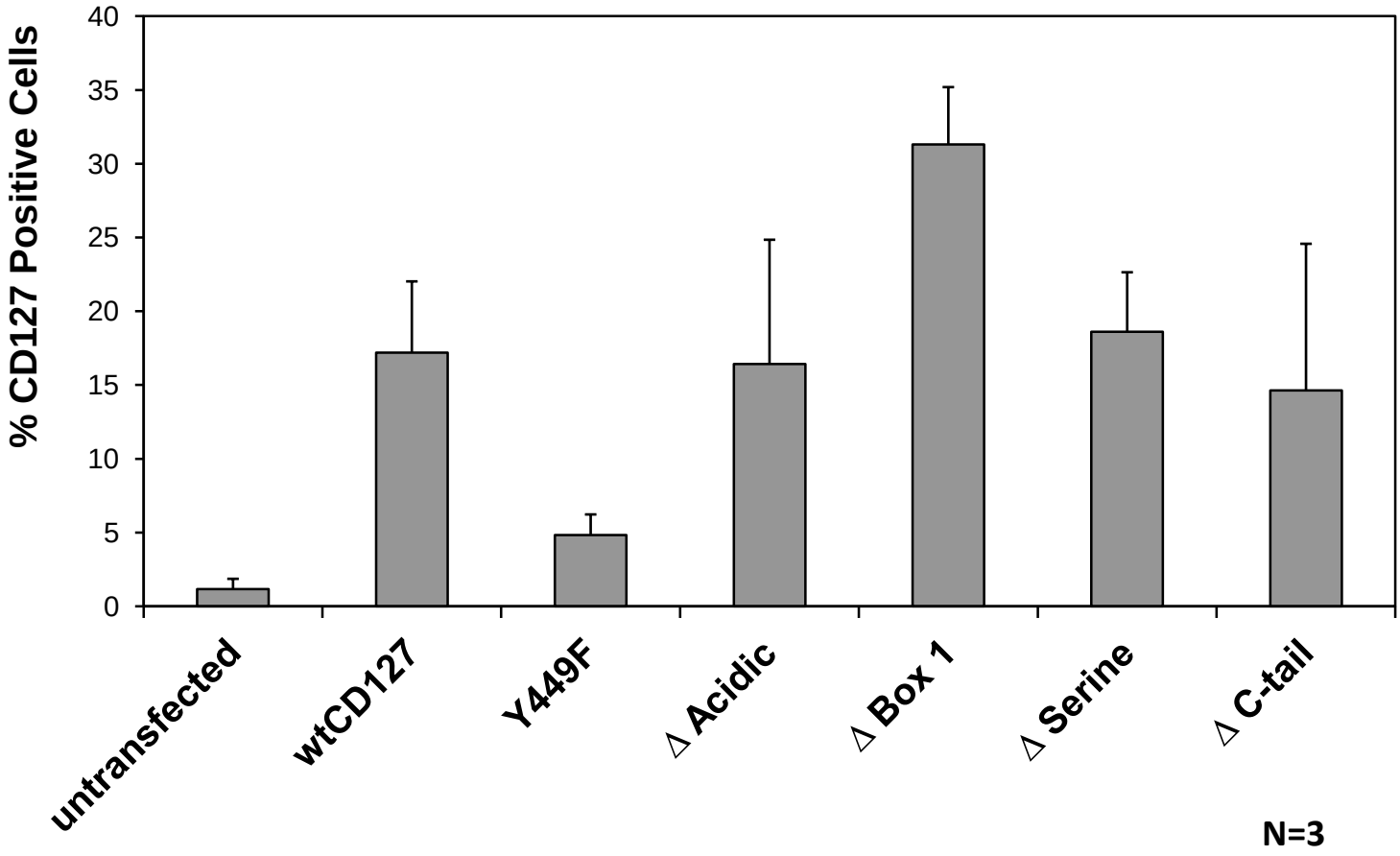
Figure 19: Percentage of Jurkat cells expressing surface CD127 at 1, 4, 8, 12 and 15 days post nucleofection. Jurkat cells were transfected with wtCD127. Error bars represent SD of two independent experiments.


25
20
15
10
5
0
% CD127 Positive Cells
1
4
8
12
15
Number of days post transfection

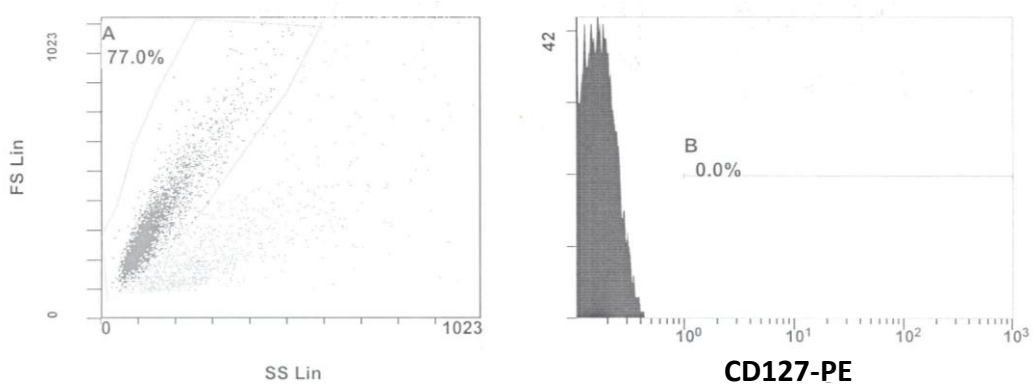
N=2

Figure 20: CD127 mutant expression on Jurkat cells. A) Percentages of Jurkat cells expressing surface CD127 at 24 hours post nucleofection. Jurkat cells were transfected with wtCD127 or deletion mutants. Error bars represent SD of three independent experiments. B) Representative flow cytometry histograms of Jurkat cells expressing surface CD127 at 24 hours post nucleofection.

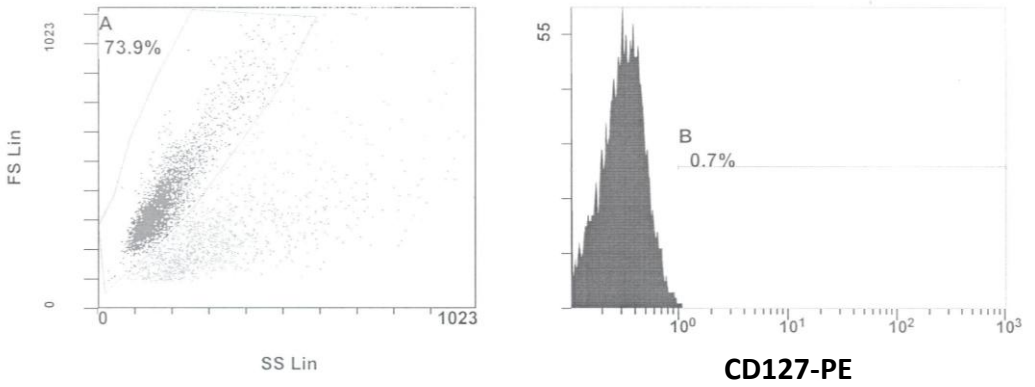
(A)


% CD127 Positive Cells
40
35
30
25
20
15
10
5
0
untransfected
wtCD127
Y449F
∆ Acidic
∆ Box 1
∆ Serine
∆ C-tail
N=3

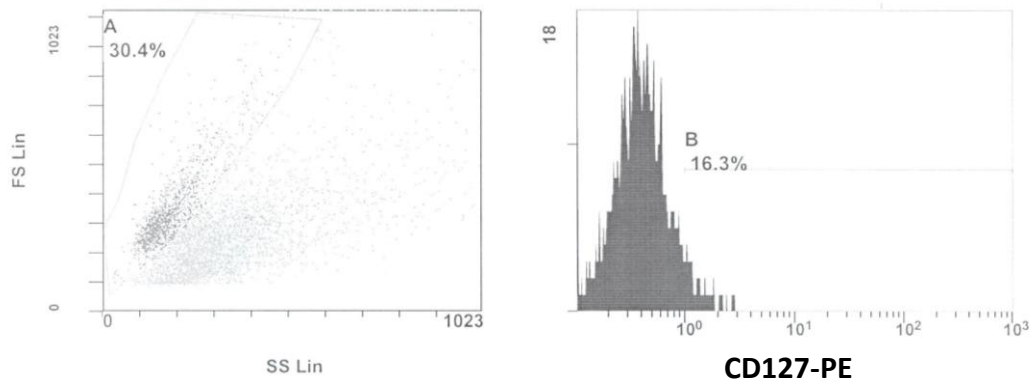
(B)



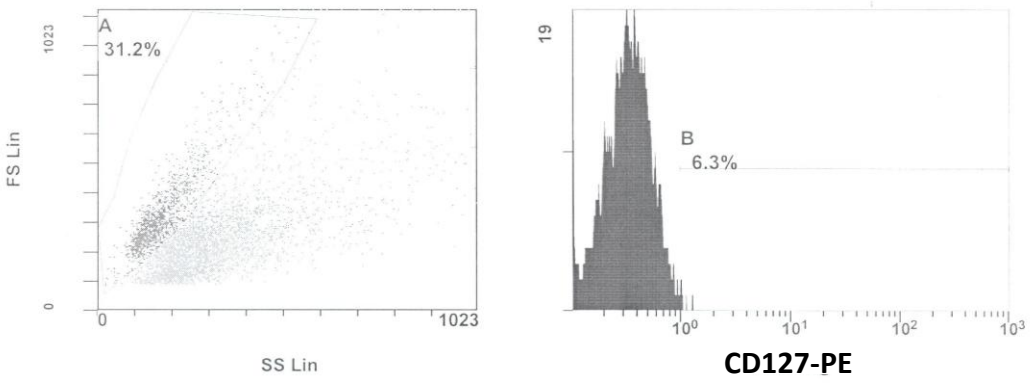
Unstained Jurkats



Untransfected Jurkats

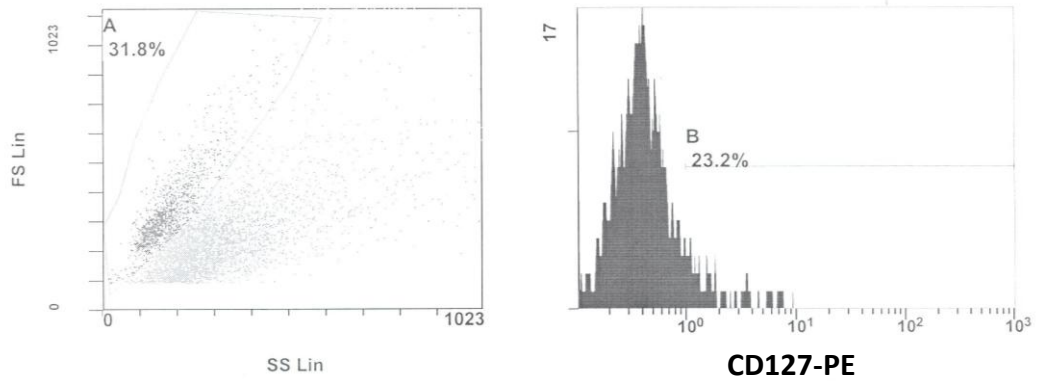


wtCD127 transfected Jurkats

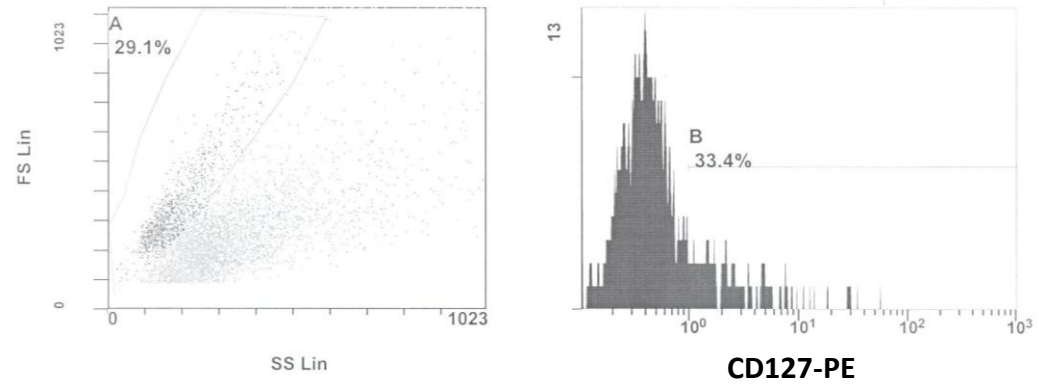


Y449F transfected Jurkats

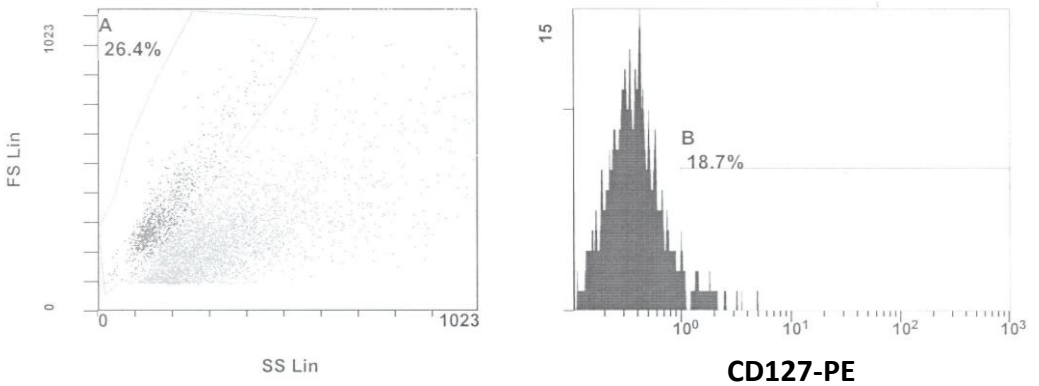
(B)



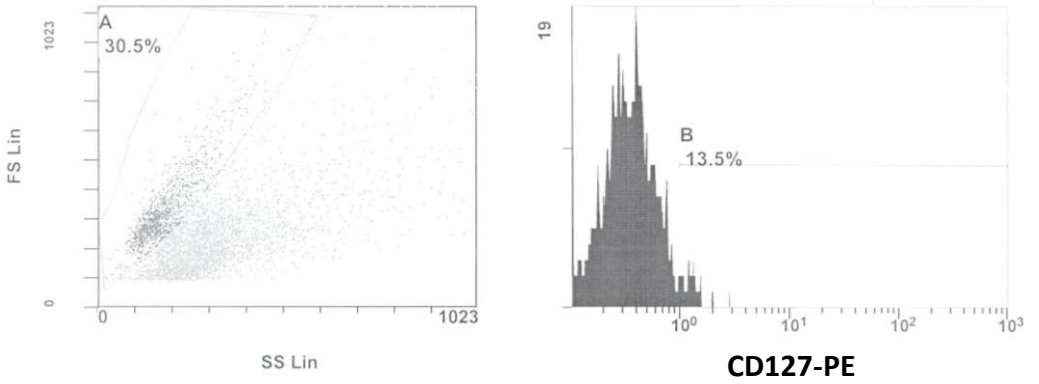
Δ Acidic domain transfected Jurkats



Δ Box1 domain transfected Jurkats



Δ Serine domain transfected Jurkats



Δ C-tail transfected Jurkats

immunoprecipitation of CD127 was attempted using wtCD127-transfected Jurkat cells. In figure 21, a CD127-Fc chimeric protein which runs at the same molecular weight as wt protein is used as a size marker. In Jurkat cells transfected with the plasmid expressing wt CD127, a faint band at the appropriate molecular weight was detected with anti-CD127 antibodies from two independent transfection experiments. This band is not seen when the immunoprecipitation was carried out with IgG isotype antibodies or in untransfected cells. The "ghost" band below CD127 likely represents antibody heavy chain from the immunoprecipitation.

I next carried out immunoprecipitations with the complete panel of CD127 mutant plasmids transfected into Jurkat cells. As seen in figure 22, CD127 protein was detected in all cases. The size of the mutant proteins relative to the chimera differed corresponding to the number of amino acids deleted. In all lanes from transfected cells, lower molecular weight bands were also immunoprecipitated with the CD127 polyclonal antibody. These may represent degraded protein fragments or unglycosylated CD127.

3.2.2 Production of pure and biologically active wild type Tat protein

3.2.2.1 Production of wild type Tat protein

pTatC6H-1 encoding for wild type HIV-1 Tat protein tagged at the C-terminus with six histidine residues was transformed into *E. coli.* The hisditine-tagged Tat protein (Tat6xHis) was captured and purified from bacterial lysates using nickel-nitriloacetic acid (Ni-NTA) columns as described in Chapter 2. Using this method, a single 14 kDa band could be purified to homogeneity and visualized by silver stain analysis (Figure 23).

Figure 21: Immunoprecipitation of CD127 protein from Jurkat cells transfected with wild type CD127. Jurkat cells were transfected with wt CD127 and lysed at 24 hours. CD127 was immunoprecipitated from lysed Jurkat cells with polyclonal goat anti-human CD127 antibodies or goat isotype as control and probed by Western for CD127 using monoclonal mouse anti-human CD127 antibody.

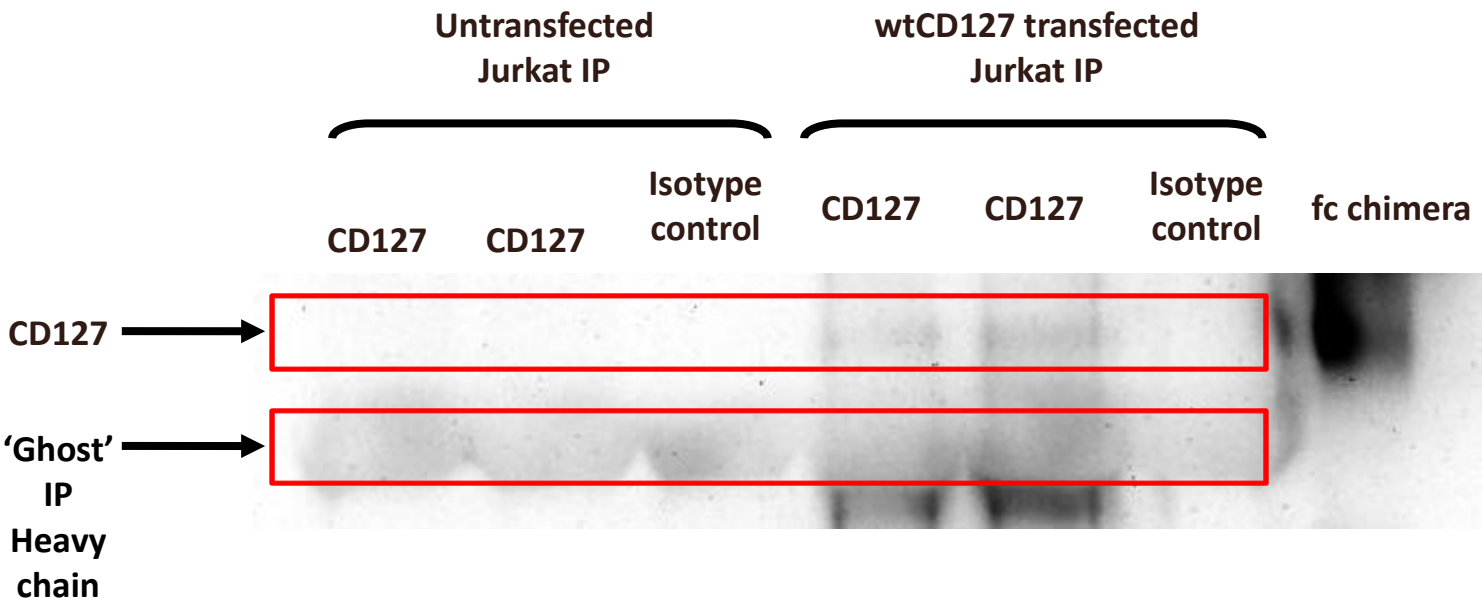

Untransfected
Jurkat IP
wtCD127 transfected
Jurkat IP
CD127
CD127
Isotype
control
CD127
CD127
Isotype
control
fc chimera
CD127
'Ghost'
IP
Heavy
chain

Figure 22: Immunoprecipitation of CD127 protein from Jurkat cells transfected with mutant constructs. Jurkat cells were transfected with mutant constructs and lysed at 24 hours. CD127 was immunoprecipitated from lysed Jurkat cells with polyclonal goat anti-human CD127 antibodies and probed by Western for CD127 using monoclonal mouse anti-human CD127 antibody.

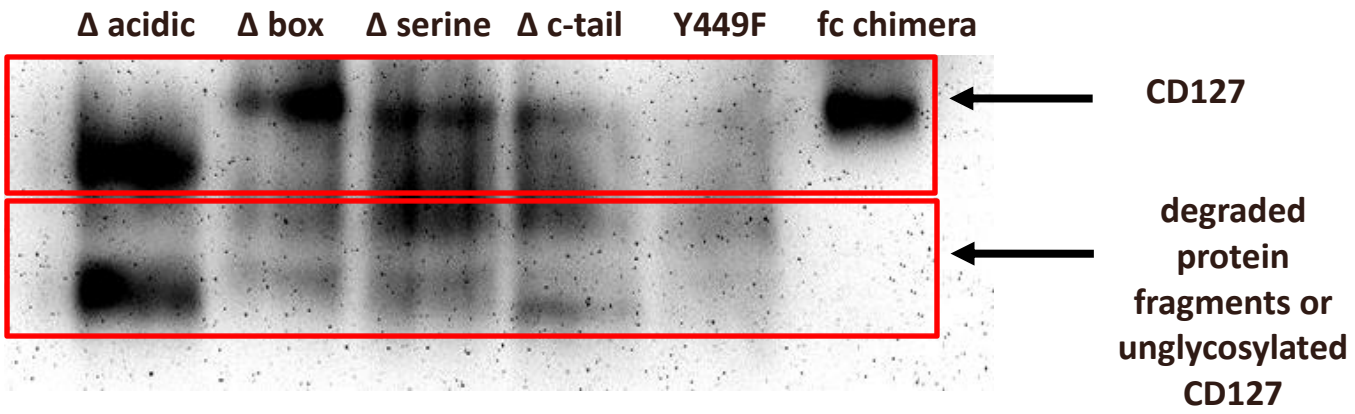
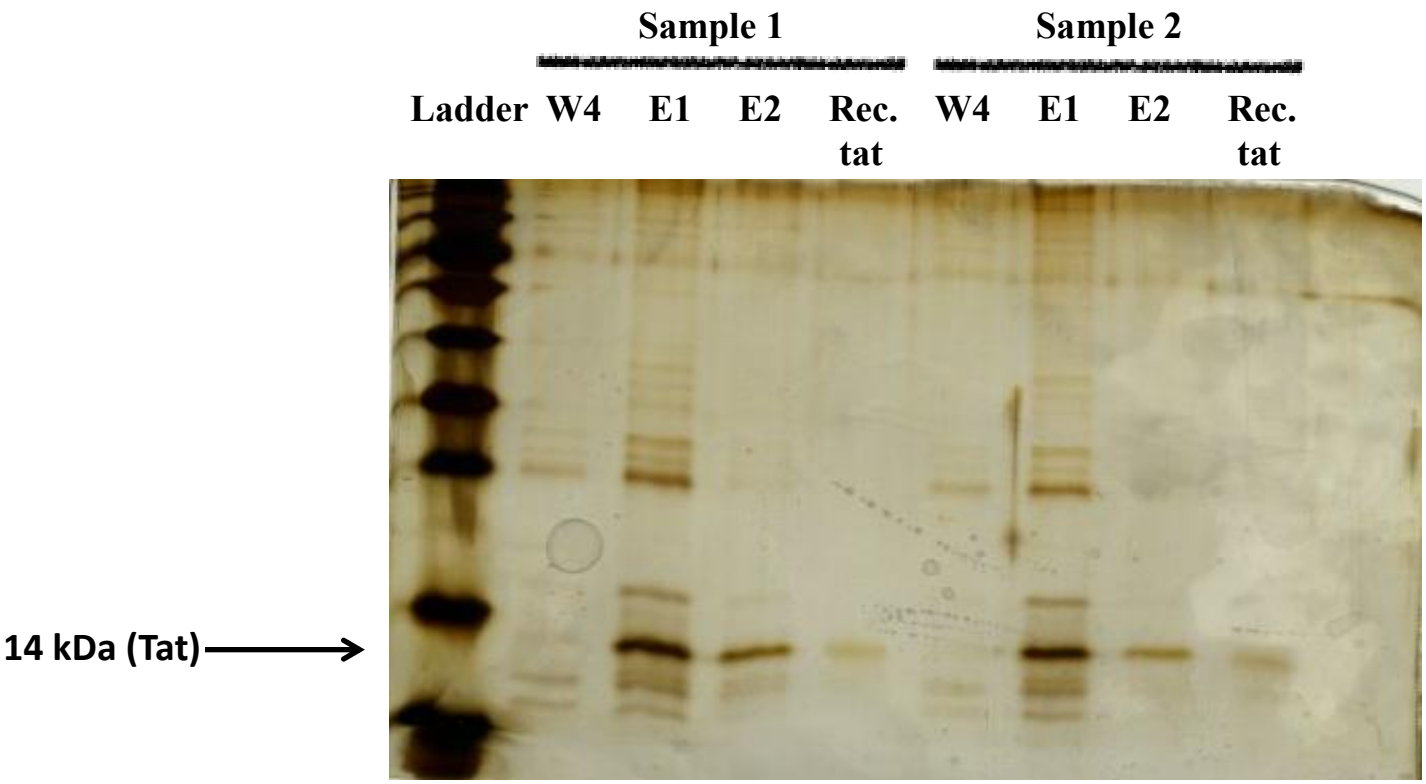

Δ acidic
Δ box
Δ serine
Δ c-tail
Y449F
fc chimera
CD127
degraded protein fragments or unglycosylated CD127

Figure 23: Silver stain analysis showing purification of recombinant wild type Tat from *E. coli* lysates using Ni-TED columns. W: wash, E: eluant. Rec.: is recombinant wild type Tat purchased from Advanced Bioscience laboratory Inc.


Sample 1
Sample 2
Ladder
W4
E1
E2
Rec. tat
W4
E1
E2
Rec. tat
14 kDa (Tat)

3.2.2.2 Identification of Tat protein

Although wild type Tat protein isolated from bacteria is known to run at 14 kDa, I sought to confirm the identity of the protein as HIV-1 Tat by Western blot using anti-Tat antibodies. As shown in figure 24, Ni-TED eluates probed with the anti-Tat antibodies identified the single 14 kDa band as HIV Tat.

3.2.3 The CD127 cytoplasmic acidic region, Box 1, Serine region and C-tail are not required for CD127/Tat Binding.

Using the CD127 mutant constructs, I next set out to determine which region(s) of CD127 are required to interact with Tat. It would have been preferred to examine the ability or the loss of the ability of Tat to downregulate CD127 mutants off the surface of transfected Jurkats by flow cytometry. Unfortunately, the CD127 mutant transfection efficiencies were too low. CD127-Tat interaction, however, was directly examined by co-immunoprecipitation. First, Jurkat cells were transfected with the plasmid encoding full length wt CD127. After 24 hours, 5x 10^6 cells were lysed in 100 μl IP lysis buffer for 1 hour. After the removal of pellet debris by centrifugation, 450 μl of Dilution buffer was added each lysate along with 1, 5 or 10 micrograms of purified Tat protein. CD127 was then immunoprecipitated using polyclonal goat anti-human CD127 antibodies and after separating by SDS-PAGE the immune complexes were probed for Tat. As seen in figure 25 and consistent with previous data from our lab, Tat was co-immunoprecipitated with CD127 protein. Incubating with 10 micrograms of Tat provided the clearest signal.

Next, Jurkat cells were transfected with the full panel of CD127 expression plasmids and after 24 hours the cells were lysed and lysates incubated with 10 micrograms purified Tat

Figure 24: Western blot analysis of purified recombinant wild type Tat isolated from *E. coli* lysates using Ni-TED columns. E: eluant and Rec.: recombinant wild type Tat purchased from Advanced Bioscience laboratory Inc.

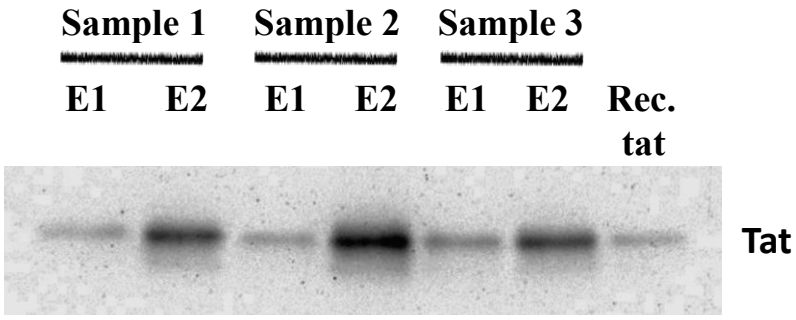
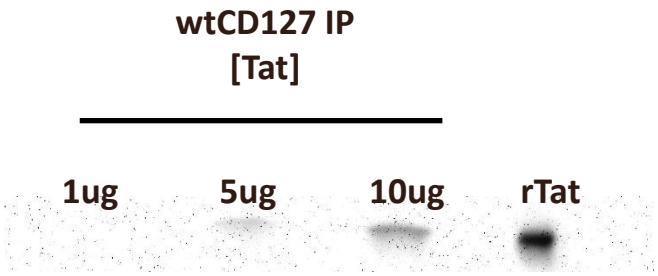
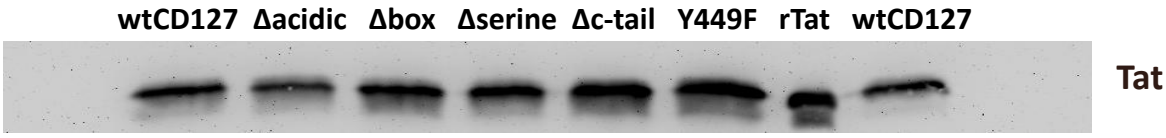

Sample 1
Sample 2
Sample 3
E1
E2
E1
E2
E1
E2
Rec. tat
Tat

Figure 25: Tat protein is co-immunoprecipitated with full length wt CD127**.** Wild type CD127 transfected Jurkat lysates were incubated with Tat protein then immunoprecipitated with anti-CD127 antibodies. Immune complexes were separated via SDS-PAGE and analyzed by Western blot for Tat. rTat: recombinant Tat protein included on the gel as a size marker.


wtCD127 IP
[Tat]
1ug
5ug
10ug
rTat

protein. After immunoprecipitating CD127, immune complexes were probed for Tat protein. Unexpectedly, Tat was immunoprecipitated with all CD127 mutants (figure 26). These data require confirmation but suggest neither tyrosine 449, box 1, the acidic region, serine region nor c-tail are specifically required for Tat binding to CD127.

Figure 26: Tat does not appear to interact with tyrosine 449, box 1, the acidic region, serine region or c-tail of the CD127 cytoplasmic domain. Jurkat cells were transfected with each of the CD127 mutant expression plasmids and then lysed at 24 hours. Lysates were incubated with Tat proteins and then immunoprecipitated with anti-CD127 antibodies. Immune complexes were separated via SDS-PAGE and analyzed by Western blot for Tat.


wtCD127
Δacidic
Δbox
Δserine
Δc-tail
Y449F
rTat
wtCD127
Tat

4. Chapter 4: Discussion

In order to investigate CD127/Tat interactions, a series of mutant CD127 proteins were produced. Molecular cloning techniques were used to generate five plasmids encoding CD127 mutants. These plasmids allowed for the expression of the full length and mutant CD127 in a eukaryotic cell line. The full length CD127 cytoplasmic tail is comprised of four well defined regions (118). I was able to create four deletion mutants, each sequentially lacking a single region of the cytoplasmic tail: Δ box1, Δ acidic, Δ serine, Δ c-tail along with a point mutation Y449F.

To continue the investigation of CD127/Tat interactions, five T lymphoblast cell lines were explored. To determine the effects of the mutant CD127, the clones generated were expressed in a cell line lacking endogenous CD127 expression. Of interest was TALL-104, an IL-2-dependent human leukemic T-cell line, which was derived from a CD8 T cell linage, while the other cell lines were derived from a CD4 T cell linage. Originally, TALL-104 was identified as the cell line of choice; it seemed most appropriate since it expressed negligible levels of CD127 mRNA and protein and it was closest to the model being replicated. The model replicated being: HIV Tat protein binds to CD127 through its cytoplasmic tail thus downregulate CD127 on CD8 T-cells and impairing their signaling and proliferation. However the TALL-104 T cell line quickly proved to be a difficult cell line to culture. Their growth was limited to specific

conditions with recombinant human IL-2 and human albumin and their doubling time was much less than that of the other T cell lines. The TALL-104 cell line had a doubling time of 10 – 12 days while the other T cell lines had a doubling time of 4 – 5 days. In the investigation to select another cell line, the Jurkat T cell line proved to have precedence over the other T cell lines examined. Alves *et. al.* was able to successfully produce a Jurkat cell line that expresses CD127 (231). These Jurkat-CD127 cells contained a retrovirally transduced CD127-IRES-GFP sequence and expressed the human CD127 cDNA from the cytomegalovirus promoter. Furthermore the Jurkat cell line showed low levels of CD127 mRNA and negligible levels of endogenous CD127 protein (Figure 17 a and b); similarly, Kim *et. al.* reported the lack constitutive CD127 expression in the Jurkat cell line (232). Therefore the Jurkat cell line served as the optimal cell line to express the mutant CD127 clones and examine their effects.

The transfection of each mutant CD127 clone into the Jurkat cell line proved to be successful where the average percent CD127-positive cells varied between 15% - 30% with the exception of the Y449F clone which showed the lowest number of CD127 positive cells at 5 ± 1% (Figure 20). It is possible that the Y449F did not transport to the cell membrane as efficiently as the wild-type. The percentage of CD127- positive cells seen here were lower than that seen in literature (95). Jiang *et.al.* showed 40% transfection efficiency for their wild type, Δ box1, Δ serine and Y449F of murine CD127 (mCD127) clones (95). It is possible that the electric shock caused low cell viability during transfection and contributed to the low transfection efficiency. The objective behind the nucleofection is to use a current pulse that will maintain a ~40-70% cell viability after electroporation. Therefore, it is possible that a lower length of pulse could have been used by lowering the capacitance to increase cell viability. Different cell lines have

different transfection capacities, and it is possible that another cell line could have yielded higher transfection efficiency.

The western blotting analysis for the presence of CD127 protein in transfected Jurkat cells proved to be below detectable limits (data not shown). Fortunately, the immunoprecipitation of CD127 from the CD127-transfected Jurkat cells allowed for the detection of CD127 (Figure 21 and 22). We suspect that the increased sensitivity of CD127 detection with immunoprecipitation was due to selection and concentration of CD127 via antibody in the cell lysate. Also consistent with the lower percent positive cells following transfection of the Y449F mutant by flow cytometry data, the Y449F mutant showed the lowest CD127 protein by Western blot (Figure 20 and 22). The strongly suggests the issue here is low transfection efficiency or protein stability and not a problem with transport of the protein to the cell surface.

Immunoprecipitation of the CD127 mutant proteins, each with a different size consistent with the number of amino acids deleted, demonstrated successful expression within Jurkat cells (Figure 22). The CD127 deletion mutants ran faster than the recombinant human CD127–Fc chimera protein which has a molecular mass of ~90 kDa (Figure 22). I believe that the heavier band in each lane is the glycosolyated form of CD127. I suspect that the lower molecular weight bands are the unglycosylated form of the protein. This finding is similar to previous descriptions in mice in which multiple bands were detected by SDS–PAGE from cell lines transfected with murine CD127. In mice, the 3 protein bands were thought to represent the unglycosylated (49.6 kDa), the glycosylated (68 kDa) and the dimeric (153 kDa) forms of the

receptor (118). Therefore, I suspect that the multiple protein bands detected in figure 23 represent the different forms of the receptor.

In this chapter, I was able to successfully produce the CD127 mutant proteins in a Jurakt cell line. Therefore, in order to characterize CD127/Tat physical interactions, large quantities of Tat protein were needed. I utilized an optimized Ni-NTA column system to isolate histidine-tagged HIV-1 Tat at a high yield and purity from *E. coli*. These proteins were stored stably at -80^{o}C.

It has been established that Tat binds specifically to the cytoplasmic tail of CD127; therefore the generated CD127 mutants each lacking a successive domain of the wild type CD127 cytoplamic tail were utilized to identify the Tat binding region. CD127-Tat interactions were examined by co-immunoprecipitation. CD127 transfected Jurkat whole cell lysates were incubated with purified Tat protein. Thereafter, CD127, and any protein bound to it, was immuneprecipitated and ran on a Western blot and probed for Tat. In this chapter, I show that each of the CD127 mutants was still able to bind and co-immunoprecipitate Tat despite the removal of each of the regions within the cytoplasmic domain. Therefore, it appears that the region needed for Tat binding was still intact. The region of Tat required for CD127 binding has already been established: being the N-terminus of Tat. The first 10 aa of Tat's N-terminal domain contains three acidic residues which are negatively charged. Given this, I originally hypothesized that CD127 acidic domain was an attractive target for Tat binding. The CD127 acidic domain contains a positively charged region (KKRIK) directly adjacent to the transmembrane domain on the cytosolic side of the protein. A second positively charged sequence, also within the acidic domain, is found 18 aa further into the tail of the receptor.

However, from the results, Tat still binds to CD127 even when the acidic region of the cytoplasmic tail was removed. The extracellular region of CD127 is well documented in the literature; however the structure of the cytoplasmic domain is not. Therefore, I suspect that Tat might bind the CD127 cytoplasmic domain through multiple regions on the folded protein. The consecutive deletions of each of the CD127 cytoplasmic domains might remove some of the Tat binding regions but still allow for partial binding of Tat. Another possible mechanism, although unlikely due to establishment of direct CD127 and Tat binding is an adaptor protein that brings Tat and CD127 together.

5. Chapter 5: Conclusion

The past few years have seen impressive progress in HIV research. While combination antiretroviral therapy has lead to significant decreases in morbidity and mortality, the economic cost and long term toxicities associated with these medications remain a challenge. Impaired cell mediated immunity is one of the clinical hallmark of HIV infection and likely contributes to the inability to clear this virus. In order to further develop novel therapeutic strategies including a cure, it is critical that we understand how HIV impairs the function of CD8 T cells.

IL-7 signalling is essential for normal CD8 T-cell development and function, and our lab (82) and others (83,85,108-110) have shown decreased expression of the IL-7 receptor alpha-chain (CD127) on circulating CD8 T-cells in HIV+ patients. This down regulation of CD127 was shown to result in poor CD8 T cell function, proliferation and perforin synthesis after stimulation with IL-7 (104,224). Fortunately, suppression of HIV replication with antiretroviral therapy was associated with an apparent partial recovery of CD127 on CD8 T cells (82). Although the exact mechanism by which CD127 is down regulated in vivo has not been definitely established, our lab has shown soluble HIV Tat protein when added to CD8 T-cells in culture is able to specifically down regulated CD127 at the cell surface. HIV Tat did not alter the surface expression of numerous other surface receptors including the common γ-chain (CD132), CD3, CD2, or CD28 (82).

HIV Tat protein is secreted by infected CD4 T cells and is taken up by neighbouring uninfected CD8 T cells by endocytosis through clathrin coated pits. As the endosomes acidify, Tat escapes to the cytosol in a pH-dependent process. Once in the cytoplasm, Tat translocates to the inner leaflet of the cell membrane where it binds to the cytoplasmic tail of CD127 and

targets the receptor to the proteasome for degradation. Our lab has previously shown that purified Tat protein physically interacts with CD127 on CD8 T cells *in vitro* (104,224). More specifically, our lab has shown that this interaction is mediated by Tat's N-terminal domain, where the removal of the N-terminus of Tat prevented Tat from co-immunoprecipitating with CD127 (225). Tat's ability to downregulate CD127 was shown to be time and dose dependent and can be blocked with anti-Tat antibodies; also this downregulation can be reversed by removing Tat from the culture media (104,224). Therefore, by binding to CD127, Tat is able to mediate the accelerated internalization and proteasomal degradation of CD127.

Tat-mediated CD127 downregulation may in part explain the loss of cell-mediated immunity seen in HIV-1 infection. By removing CD127 from the cell surface, the HIV Tat protein is able to reduce IL-7 signalling and impair CD8 T-cell proliferation and function. Therefore, to better understand the molecular details of CD127/Tat interactions, I have set out to determine the region(s) in the cytoplasmic domain of CD127 that are required for its interaction with Tat. This work could lead to the development of novel drugs to target and disrupt the CD127/Tat interaction possibly allowing restoration of CD127 on the cell surface and improved IL-7 signalling.

To determine which domain(s) in the cytoplamic tail of CD127 are required for interaction with Tat, a series of plasmids encoding for CD127 deletion mutants (see figure 15) were successfully created. This series of mutant CD127 coding sequences were transfected into a eukaryotic expression system, the Jurakt cell line, and CD127 proteins were successfully expressed. Before determine which region on CD127 is required for Tat binding, an optimized

Ni-NTA column system was used to successfully isolate histidine-tagged HIV-1 Tat at a high yield and purity from *E. coli.* This HIV Tat protein was used to treat the lysates of the Jurakt cells transfected with the panel of CD127 mutants. CD127 was then immunoprecipitated, followed by Western analysis of the immune complexes to detect Tat protein. Unexpectedly, Tat was immunoprecipitated with all CD127 mutants (figure 26). This suggests neither tyrosine 449, box 1, the acidic region, serine region nor C-tail are specifically required for Tat binding to CD127.

The three-dimensional structure of the extracellular domain of CD127 is well documented in the literature; however the structure of the cytoplasmic domain is not. I suspect that Tat might bind the CD127 cytoplasmic domain through multiple regions on the folded protein. The successive deletion of each of the identified regions within the CD127 cytoplasmic domain might remove some of the Tat binding regions but still allow for partial binding of Tat.

This works serves to add an important piece to the puzzle examining CD127/Tat interactions and feeds into the mechanism by which Tat down regulates CD127 protein from the surface of CD8 T cells. The created series of CD127 mutants can be used to generate stable cell lines expressing the different CD127 isoforms on their cell surface. These stable cell lines can then be used to determine the region(s) on CD127 required for Tat induced down regulation. In other words, Tat may bind to several places on the CD127 cytoplasmic tail but binding to one region may be functionally relevant to induce receptor internalization and degradation. Our lab has now recently shown the HIV Tat protein binds to cytokine-inducible SH2-containing (CIS) protein through its basic domain (225). Of particular interest, CIS was shown to associate with CD127 in response to IL-7, and that this interaction is dependent on CD127 Y449 phosphorylation and Jak1/STAT5 signalling (111). CIS protein is known to

negatively regulate and inhibit signalling by binding to receptors and blocking STAT binding and by forming a complex with the receptor and recruiting ubiquitin ligases (113,117,233). Although definitive proof is still required, we suggest Tat can act as an adaptor protein to recruit CIS to the receptor and therefore direct CD127 to the proteasome for degradation (225). The mechanism by which CIS interacts with both CD127 and Tat is yet to be explored.

Since HIV Tat protein is able to reduce IL-7 signalling and impair CD8 T-cell proliferation and function by removing CD127 from the cell surface, attempts to possibly disrupt Tat binding to CD127 may prove a fruitful avenue for novel treatment. In an indirect method, Tat-targeting therapies could open the possibility of restoring CD127 expression and IL-7 signaling in CD8 T-cells leading to improved cell mediated immunity in HIV+ individuals.

Cross-sectional and longitudinal studies have indicated a correlation between anti-Tat humoral (234,235) and cell-mediated immune responses (236,237) and asymptomatic infection and slower disease progression (236,237). A stronger anti-Tat immune response has been seen in both long-term non-progressors and slow progressors (236,237). Furthermore, CD8+T cell responses against Tat correlate with an earlier control of initial HIV acute infection in both humans and experimental animals (238-240). With these observations in mind, disrupting or sequestering Tat could prove to be of great therapeutic benefit.

HIV Tat protein is produced very early during infection and is secreted from infected cells. Given this information and the strong correlation between anti-Tat immunity and decreased disease progression, Tat has become an important vaccine candidate for humoral and cell mediated immunity. Indeed, preclinical as well as phase I and phase II clinical trials

investigating Tat as a preventative or therapeutic vaccine are well on their way. The results from phase I trials (241-244) and preclinical studies (240,245,246) indicate that administration of the Tat vaccine is safe, well tolerated and immunogenic. In these studies, Tat vaccination induced strong and durable humoral and cellular immune responses in all vaccinees. And remarkably, vaccination in these studies elicited a wide repertoire of B-cell responses directed at Tat, with an effective, statistically significant induction of Tat-specific neutralizing antibodies. While these responses are rarely seen in natural infection (241,247-249), they do correlate significantly with slow progression to disease (234).

In a phase II multicenter open label clinical trial (250), immunization of HIV+ individuals with Tat was shown to be safe and immunogenic, and lead to the reversion of some immunological abnormalities present during HIV infection (251-254). Out of the three vaccinee groups, anti-Tat antibody negative, HAART-treated and ART non-responders, the immune comprised ART non-responders showed the greatest therapeutic effects (250). In particular, the frequency and number of T-reg lymphocytes, reduced during HIV infection, were stably increased upon Tat immunization. Tat vaccination also resulted in an increase in CD4 and CD8 T cell responses against the HIV Env and Tat antigens (250).

As impaired cell mediated immunity is the clinical hallmark of HIV infection, proper CD8 T cell mediated immune responses could potentially control the infection. If the CD127/Tat interactions could be targeted and prevented, it is possible sustained CD127 expression on CD8 T cells would allow the individual's immune system to maintain strong functional CTL responses during HIV-1 infection. Ensuring proper IL-7 signalling would also be expected to help regulate T cell homeostasis and establish memory T cells. Therefore, Tat-targeting therapeutics could

potentially negate the Tat-mediated CD127 interference and sustain higher CD127 expression. This could allow for a sustained and healthy CD8 T cell-mediated immune response to eradicate the infection and potentially favour immune clearance of the virus.

6. References

(1) Montagnier L. Historical essay. A history of HIV discovery. Science 2002;298(5599):1727-8.

(2) Moss AR, Bacchetti P. Natural history of HIV infection. AIDS 1989;3(7):A100.

(3) Gallo RC. Historical essay. The early years of HIV/AIDS. Science 2002;298(5599):1728-30.

(4) Centres for Disease Control. Human Immunodefiency Virus in the United States. MMWR 1988;36(5-6):15.

(5) Osmond D, Moss AR. The prevalence of HIV infection in the United States, a revision of the CDC estimates. AIDS Clinical Review 1989.

(6) Watts JM, Dang KK, Gorelick RJ, Leonard CW, Bess JW Jr, Swanstrom R, et al. Architecture and secondary structure of an entire HIV-1 RNA genome. Nature 2009;460(7256):711-6.

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

(8) Granich R, Crowley S, Vitoria M, Smyth C, Kahn JG, Bennett R, et al. Highly active antiretroviral treatment as prevention of HIV transmission: review of scientific evidence and update. Curr Opin HIV AIDS 2010;5(4):298-304.

(9) Wu L, Gerard NP, Wyatt R, Choe H, Parolin C, Ruffing N, et al. CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5. Nature 1996;384(6605):179-83.

(10) Freed EO. Review HIV-1 replication. Somat Cell Mol Genet. 2001;26(1-6):13-33.

(11) Robinson HL. New hope for an AIDS vaccine. Nat Rev Immunol. 2002;2(4):239-50.

(12) Menéndez-Arias L. Molecular basis of fidelity of DNA synthesis and nucleotide specificity of retroviral reverse transcriptases. Prog Nucleic Acid Res Mol Biol. 2002;71:91-147.

(13) Svarovskaia ES, Cheslock SR, Zhang WH, Hu WS, Pathak VK. Retroviral mutation rates and reverse transcriptase fidelity. Front Biosci 2003;8:117-34.

(14) Schröder AR, Shinn P, Chen H, Berry C, Ecker JR, Bushman F. HIV-1 integration in the human genome favors active genes and local hotspots. Cell. 2002;110(4):521-9.

(15) Pluymers W, De Clercq E, Debyser Z. HIV-1 Integration as a Target for Antiretroviral Therapy: A Review. Curr Drug Targets Infect Disord. 2001;1(2):133-49.

(16) Karn J. Tackling Tat. J Mol Biol 1999;293(2):235-54.

(17) Gottfredsson M, Bohjanen PR. Human immunodeficiency virus type I as a target for gene therapy. Front Biosci 1997;2(d):619-34.

(18) Coiras M, López-Huertas MR, Sánchez del Cojo M, Mateos E, Alcamí J. Dual Role of Host Cell Factors in HIV-1 Replication. AIDS Rev. 2010;12(2):103-12.

(19) Hiscott J, Kwon H, Génin P. Hostile takeovers: viral appropriation of the NF-kB pathway. J Clin Invest. 2001;107(2):143-51.

(20) Johri MK, Mishra R, Chhatbar C, Unni SK, Singh SK. Tits and bits of hiv tat protein. Expert Opin Biol Ther 2011;11(3):269-83.

(21) Grulich AE, Wan X, Law MG, Coates M, Kaldor JM. Risk of cancer in people with AIDS. AIDS 1999;13(7):839-43.

(22) McMichael AJ, Borrow P, Tomaras GD, Goonetilleke N, Haynes FB. The immune response during acute HIV-1 infection: clues for vaccine development. Nat Rev Immunol 2010;10(1):11-23.

(23) McMichael AJ, Rowland-Jones SL. Cellular immune responses to HIV. Nature 2001;410:980-87.

(24) Stevenson M. Dissecting HIV-1 through RNA interference. Nat Rev Immunol. 2003;3(11):851-8.

(25) Rosenberg ES, Altfeld M, Poon SH, Phillips MN, Wilkes BM, Eldridge RL, et al. Immune control of HIV-1 after early treatment of acute infection. Nature 2000;407(523):26.

(26) Zhang D, Pasternack MS, Beresford PJ, Wagner L, Greenberg AH, Lieberman J. Induction of rapid histone degradation by the cytotoxic T lymphocyte protease Granzyme A. J Biol Chem. 2001;276(5):3683-90.

(27) Metkar SS, Wang B, Ebbs ML, Kim JH, Lee YJ, Raja SM, et al. Granzyme B activates procaspase-3 which signals a mitochondrial amplification loop for maximal apoptosis. J Cell Biol. 2003;160(6):875-85.

(28) Pinkoski MJ, Waterhouse NJ, Heibein JA, Wolf BB, Kuwana T, Goldstein JC, et al. Granzyme B-mediated apoptosis proceeds predominantly through a Bcl-2-inhibitable mitochondrial pathway. J Biol Chem. 2001;276(15):12060-7.

(29) Chávez-Galán L, Arenas-Del Angel MC, Zenteno E, Chávez R, Lascurain R. Cell death mechanisms induced by cytotoxic lymphocytes. Cell Mol Immunol. 2009;6(1):15-25.

(30) Li H, Zhu H, Xu CJ, Yuan J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell. 1998;94(4):491-501.

(31) Ogg GS, Jin X, Bonhoeffer S, Dunbar PR, Nowak MA, Monard S, et al. Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma viral RNA load. Science 1998;279(5359):2103-6.

(32) Salazar-Gonzalez JF, Salazar MG, Keele BF, Learn GH, Giorgi EE, Li H, et al. Genetic identity, biological phenotype, and evolutionary pathways of transmitted/founder viruses in acute and early HIV-1 infection. J Exp Med 2009;206(6):1273-89.

(33) Bernardin F, Kong D, Peddada L, Baxter-Lowe LA, Delwart E. human immunodeficiency virus mutations during the first month of infection are preferentially found in known cytotoxic t lymphocyte epitopes. J Virol 2005;79(17):11523-8.

(34) Altfeld M, Kalife ET, Qi Y, Streeck H, Lichterfeld M, Johnston MN, et al. HLA Alleles Associated with Delayed Progression to AIDS Contribute Strongly to the Initial CD8(+) T Cell Response against HIV-1. PLoS Med 2006;3(10):e403.

(35) Carrington M, O'Brien SJ. The influence of HLA genotype on AIDS. Annu Rev Med 2003;54:535-51.

(36) Sharma B, Gupta S. Antigen-specific primary cytotoxic T lymphocyte (CTL) responses in acquired immune deficiency syndrome (AIDS) and AIDS-related complexes (ARC). Clin Exp Immunol 1985;62(2):296-303.

(37) Bettens F, Pichler CE, Herrmann B, de Weck AL, Pichler WJ. Selective stimulation of CD4+ versus CD8+ T-cell subsets in symptomatic and asymptomatic HIV-1-infected individuals. AIDS Res Hum Retroviruses 1991;7(9):773-80.

(38) Gerstoft J, Dickmeiss E, Mathiesen L. Cytotoxic capabilities of lymphocytes from patients with the acquired immunodeficiency syndrome. Scand J Immunol 1985;22(5):463-70.

(39) Miedema F, Petit AJ, Terpstra FG, Schattenkerk JK, de Wolf F, Al BJ, et al. Immunological abnormalities in human immunodeficiency virus (HIV)-infected asymptomatic homosexual men. HIV affects the immune system before CD4+ T helper cell depletion occurs. J Clin Invest 1988;82(6):1980-14.

(40) Feller L, Lemmer J. Aspects of immunopathogenic mechanisms of HIV infection. SADJ 2007;62(10):432-6.

(41) van Baarle D, Hovenkamp E, Callan MF, Wolthers KC, Kostense S, Tan LC, et al. Dysfunctional Epstein-Barr virus (EBV)-specific CD8(+) T lymphocytes and increased EBV

load in HIV-1 infected individuals progressing to AIDS-related non-Hodgkin lymphoma. Blood 2001;98(1):146-55.

(42) Altfeld M, Rosenberg ES, Shankarappa R, Mukherjee JS, Hecht FM, Eldridge RL, et al. Cellular immune responses and viral diversity in individuals treated during acute and early HIV-1 infection. J Exp Med 2001;193(2):169-80.

(43) Migueles SA, Osborne CM, Royce C, Compton AA, Joshi RP, Weeks KA, et al. Lytic granule loading of CD8+ T cells is required for HIV-infected cell elimination associated with immune control. Immunity 2008;29(6):1009-21.

(44) Andersson J, Kinloch S, Sönnerborg A, Nilsson J, Fehniger TE, Spetz AL, et al. Low levels of perforin expression in CD8+ T lymphocyte granules in lymphoid tissue during acute human immunodeficiency virus type 1 infection. J Infect Dis 2002;185(9):1355-8.

(45) Fry T, Mackall C. The Many Faces of IL-7: From Lymphopoiesis to Peripheral T Cell Maintenance. The Journal of Immunology 2005;174(11):6571-76.

(46) Namen AE, Lupton S, Hjerrild K, Wignall J, Mochizuki DY, Schmierer A, et al. Stimulation of B-cell progenitors by cloned murine interleukin-7. Nature 1988;333(6173):571-3.

(47) Namen AE, Schmierer AE, March CJ, Overell RW, Park LS, Urdal DL, et al. B cell precursor growth-promoting activity. Purification and characterization of a growth factor active on lymphocyte precursors. J Exp Med. 1988;167(3):988-1002.

(48) Peschon JJ, Morrissey PJ, Grabstein KH, Ramsdell FJ, Maraskovsky E, Gliniak BC, et al. Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. J Exp Med 1994;180:1955-1960.

(49) von Freeden-Jeffry U, Vieira P, Lucian LA, McNeil T, Burdach SE, Murray R. Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. J Exp Med 1995;181:1519-1526.

(50) Puel A, Ziegler SF, Buckley RH, Leonard WJ.
Defective IL7R expression in T(-)B(+)NK(+) severe combined immunodeficiency. . Nat Genet 1998;20:394-397.

(51) Durum SK, Candèias S, Nakajima H, Leonard WJ, Baird AM, Berg LJ, et al. Interleukin 7 receptor control of T cell receptor gene rearrangement: role of receptor-associated chains and locus accessibility. J. Exp. Med. 1998;188(12):2233-2241.

(52) Corcoran AE, Riddell A, Krooshoop D, Venkitaraman AR. Impaired immunoglobulin gene rearrangement in mice lacking the IL-7 receptor. Nature 391, 904–907 (1998). 1998;391(6670):904-907.

(53) Brugnera E, Bhandoola A, Cibotti R YQ, Guinter TI, Yamashita Y, Sharrow SO, et al. Coreceptor reversal in the thymus: signaled $CD4^+8^+$ thymocytes initially terminate CD8 transcription even when differentiating into $CD8^+$ T cells. Immunity 2000;13(1):59-71.

(54) Khaled AR, Bulavin DV, Kittipatarin C, Li WQ, Alvarez M, Kim K, et al. Cytokine-driven cell cycling is mediated through Cdc25A. J. Cell Biol. 2005;169(5):755-763.

(55) Li WQ, Jiang Q, Aleem E, Kaldis P, Khaled AR, Durum SK. IL-7 promotes T cell proliferation through destabilization of p27Kip1. J. Exp. Med. 2006;203(3):573-582.

(56) Schluns K. S., Kieper W. C., Jameson S. C., Lefrancois L. Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells *in vivo*. Nature Immunol. 2000;1:426-432.

(57) Kondrack RM, Harbertson J, Tan JT, McBreen ME, Surh CD, Bradley LM. Interleukin 7 regulates the survival and generation of memory CD4 cells. J. Exp. Med. 2003;198(12):1797-1806.

(58) Jiang Q, Li W, Aiello F, Mazzucchelli R, Asefa B, Khaled A, et al. Cell biology of IL-7, a key lymphotrophin. Cytokine Growth Factor Rev. 2005;16(4-5):513-33.

(59) Aiello FB, Keller JR, Klarmann KD, Dranoff G, Mazzucchelli R, Durum SK. IL-7 induces myelopoiesis and erythropoiesis. J Immunol 2007;178:1553-1563.

(60) Sawa Y, Arima Y, Ogura H, Kitabayashi C, Jiang JJ, Fukushima T, et al. Hepatic interleukin-7 expression regulates T cell responses. Immunity 2009;30:447-457.

(61) Fry TJ, Connick E, Falloon J, Lederman MM, Liewehr DJ, Spritzler J, et al. A potential role for interleukin-7 in T-cell homeostasis. Blood 2001;97:2983-2990.

(62) Sasson SC, Zaunders JJ, Kelleher AD. The IL-7/IL-7 receptor axis: understanding its central role in T-cell homeostasis and the challenges facing its utilization as a novel therapy. Curr Drug Targets 2006;7:1571-82.

(63) Ariel A, Hershkoviz R, Cahalon L, Williams DE, Akiyama SK, Yamada KM, et al. Induction of T cell adhesion to extracellular matrix or endothelial cell ligands by soluble or matrix-bound interleukin-7. Eur J Immunol 1997;27:2562-70.

(64) Clarke D, Katoh O, Gibbs RV, Griffiths SD, Gordon MY. Interaction of interleukin 7 (IL-7) with glycosaminoglycans and its biological relevance. Cytokine 1995;7:325-30.

(65) Appasamy P.M. Interleukin-7 and Lymphopoiesis: Biological and Clinical Implications; In: Kurzrock, Razelle, Talpaz, Moshe, editor. Cytokines: Interleukins and Their Receptors. Cancer Treatment and Research, Vol. 80 ed.: Springer; 1996. p. 101-119.

(66) Sudo T, Ito M, Ogawa Y, Iizuka M, Kodama H, Kunisada T, et al. Interleukin 7 production and function in stromal cell-dependent B cell development. J Exp Med. 1989;170(1):333-8.

(67) Witte PL, Frantsve LM, Hergott M, Rahbe SM. Cytokine production and heterogeneity of primary stromal cells that support B lymphopoiesis. Eur J Immunol. 1993;23(8):1809-17.

(68) Gunji Y, Sudo T, Suda J, Yamaguchi Y, Nakauchi H, Nishikawa S, et al. Support of early B-cell differentiation in mouse fetal liver by stromal cells and interleukin-7. Blood. 1991;77(12):2612-7.

(69) Goodwin RG, Lupton S, Schmierer A, Hjerrild KJ, Jerzy R, Clevenger W, et al. Human interleukin 7: molecular cloning and growth factor activity on human and murine B-lineage cells. Proc Natl Acad Sci USA. 1989;86(1):302-6.

(70) Wiles MV, Ruiz P, Imhof BA. Interleukin-7 expression during mouse thymus development. Eur J Immunol. 1992;22(4):1037-42.

(71) Watanabe M, Ueno Y, Yajima T, Iwao Y, Tsuchiya M, Ishikawa H, et al. Interleukin 7 is produced by human intestinal epithelial cells and regulates the proliferation of intestinal mucosal lymphocytes. J Clin Invest. 1995;95(6):2945-53.

(72) Wiles MV, Ruiz P, Imhof BA. Interleukin-7 expression during mouse thymus development. Eur J Immunol 1992;22:1037-42.

(73) Golden-Mason L, Kelly AM, Traynor O, McEntee G, Kelly J, Hegarty JE, et al. Expression of interleukin 7 (IL-7) mRNA and protein in the normal adult human liver: implications for extrathymic T cell development. Cytokine. 2001;14(3):143-51.

(74) Heufler C, Topar G, Grasseger A, Stanzl U, Koch F, Romani N, et al. Interleukin 7 is produced by murine and human keratinocytes. J Exp Med. 1993;178(3):1109-14.

(75) Madrigal-Estebas L, McManus R, Byrne B, Lynch S, Doherty DG, Kelleher D, et al. Humansmallintestinalepithelialcellssecreteinterleukin-7 and differentially express two different interleukin-7 mRNA Transcripts: implications for extrathymicT-celldifferentiation. Hum Immunol. 1997;58(2):83-90.

(76) Link A, Vogt TK, Favre S, Britschgi MR, Acha-Orbea H, Hinz B, et al. Fibroblastic retiuclar cells in lymph nodes regulate the homeostatis of naive t cells. Nat Immunol. 2007;8(11):1255-65.

(77) Sorg RV, McLellan AD, Hock BD, Fearnley DB, Hart DN. Human dendritic cells express functional interleukin-7. Immunobiology. 1998;198(5):514-26.

(78) de Saint-Vis B, Fugier-Vivier I, Massacrier C, Gaillard C, Vanbervliet B, Aït-Yahia S, et al. The cytokine profile expressed by human dendritic cells is dependent on cell subtype and mode of activation. J Immunol. 1998;160(4):1666-76.

(79) Llano A, Barretina J, Gutiérrez A, Blanco J, Cabrera C, Clotet B, et al. Interleukin-7 in plasma correlates with CD4 T-cell depletion and may be associated with emergence of syncytium-inducing variants in human immunodeficiency virus type 1-positive individuals. J Virol 2001;75(21):10319-25.

(80) Rethi B, Fluur C, Atlas A, Krzyzowska M, Mowafi F, Grützmeier S, et al. Loss of IL-7Ralpha is associated with CD4 T-cell depletion, high interleukin-7 levels and CD28 down-regulation in HIV infected patients. AIDS 2005;19(18):2077-86.

(81) Rose T, Lambotte O, Pallier C, Delfraissy JF, Colle JH. Identification and biochemical characterization of human plasma soluble IL-7R: lower concentrations in HIV-1-infected patients. J Immunol. 2009 Jun 15;182(12):7389-97. doi: 10.4049/jimmunol.0900190. 2009;182(12):7389-97.

(82) MacPherson PA, Fex C, Sanchez-Dardon J, Hawley-Foss N, Angel JB. Interleukin-7 receptor expression on CD8(+) T cells is reduced in HIV infection and partially restored with effective antiretroviral therapy. J Acquir Immune Defic Syndr 2001;28(5):454-457.

(83) Colle JH, Moreau JL, Fontanet A, Lambotte O, Joussemet M, Delfraissy JF, et al. CD127 expression and regulation are altered in the memory CD8 T cells of HIV-infected patients--reversal by highly active anti-retroviral therapy (HAART). Clin Exp Immunol. 2006;143(3):398-403.

(84) Koesters SA, Alimonti JB, Wachihi C, Matu L, Anzala O, Kimani J, et al. IL-7Ralpha expression on CD4(+) T lymphocytes decreases with HIV disease progression and inversely correlates with immune activation. Eur J Immunol 2006;36(2):336-44.

(85) Paiardini M, Cervasi B, Albrecht H, Muthukumar A, Dunham R, Gordon S, et al. Loss of CD127expression defines an expansion of effector CD8+ T cells in HIVinfected individuals. J Immunol 2005;174(5):2900-9.

(86) Boutboul F, Puthier D, Appay V, Pellé O, Ait-Mohand H, Combadière B, et al. Modulation of interleukin-7 receptor expression characterizes differentiation of CD8T cells specific for HIV, EBV and CMV. AIDS 2005;19(17):1981-6.

(87) Koesters SA, Alimonti JB, Wachihi C, Matu L, Anzala O, Kimani J, et al. IL-7Ralpha expression on CD4+ T lymphocytes decreases with HIV disease progression and inversely correlates with immune activation. Eur J Immunol 2006;36(2):336-44.

(88) Binette J, Dubé M, Mercier J, Halawani D, Latterich M, Cohen EA. Requirements for the selective degradation of CD4 receptor molecules by the human immunodeficiency virus type 1 Vpu protein in the endoplasmic reticulum. Retrovirology 2007;4(75).

(89) Fluur C, De Milito A, Fry TJ, Vivar N, Eidsmo L, Atlas A, et al. Potential role for IL-7 in Fas-mediated T cell apoptosis during HIV infection. J Immunol 2007;178(8):5340-50.

(90) Napolitano LA, Grant RM, Deeks SG, Schmidt D, De Rosa SC, Herzenberg LA, et al. Increased production of IL-7 accompanies HIV-1-mediated T-cell depletion: implications for T-cell homeostasis. Nat Med 2001;7(1):73-9.

(91) Alves NL, Arosa FA, van Lier RA. Common gamma chain cytokines: dissidence in the details. Immunology letters 2007;108:113-20.

(92) Peschon JJ, Gliniak BC, Gliniak BC, Park LS, Ziegler SF, Williams DE, et al. Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. J Exp Med 1994;180:1955-60.

(93) DiSanto JP, Muller W, Guy-Grand D, Fischer A, Rajewsky K. Lymphoid development in mice with a targeted deletion of the interleukin 2 receptor gamma chain. Proc Natl Acad Sci USA 1995;92:377-81.

(94) Cao X, Shores EW, Hu-Li J, Anver MR, Kelsall BL, Russell SM, et al. Defective lymphoid development in mice lacking expression of the common cytokine receptor gamma chain. Immunity 1995;2:223-38.

(95) Jiang Q, Li WQ, Hofmeister RR, Young HA, Hodge DR, Keller JR, et al. Distinct regions of the interleukin-7 receptor regulate different Bcl2 family members. Mol Cell Biol. 2004;24(14):6501-13.

(96) Benbernou N, Muegge K, Durum SK. Interleukin (IL)-7 induces rapid activation of Pyk2, which is bound to Janus kinase 1 and IL-7Ralpha. The Journal of biological chemistry 2000;275:7060-65.

(97) Boussiotis VA, Barber DL, Nakarai T, Freeman GJ, Gribben JG, Bernstein GM, et al. Prevention of T cell anergy by signaling through the gamma c chain of the IL-2 receptor. Science 1994;266:1039-42.

(98) Giliani S, Mori L, de Saint Basile G, Le Deist F, Rodriguez-Perez C, Forino C, et al. Interleukin-7 receptor alpha (IL-7Ralpha) deficiency: cellular and molecular bases. Analysis of clinical, immunological, and molecular features in 16 novel patients. Immunol Rev 2005;203:110-126.

(99) Mazzucchelli R, Durum S. Interleukin-7 receptor expression intelligent design. Nat Rev Immunol. 2007;7(2):144-54.

(100) Park JH, Yu Q, Erman B, Appelbaum JS, Montoya-Durango D, Grimes HL, et al. Suppression of IL7Rα transcription by IL-7 and other prosurvival cytokines: a novel mechanism for maximizing IL-7- dependent T cell survival. Immunity 2004;21(2):289-302.

(101) Munitic I, Williams JA, Yang Y, Dong B, Lucas PJ, El Kassar N, et al. Dynamic regulation of il-7 receptor expression is required for normal thymopoiesis. Blood 2004;104(12):4165-72.

(102) Huster KM, Busch V, Schiemann M, Linkemann K, Kerksiek KM, Wagner H, et al. Selective expression of IL-7 receptor on memory T cells identifies early CD40L-dependent generation of distinct CD8+ memory T cell subsets. Proc Natl Acad Sci USA 2004;101(15):5610-5.

(103) Reche PA, Soumelis V, Gorman DM, Clifford T, Liu M, Travis M, et al. Human thymic stromal lymphopoietin preferentially stimulates myeloid cells. J Immunol 2001;167:336-43.

(104) Faller EM, Sugden SM, McVey MJ, Kakal JA, MacPherson PA. Soluble HIV Tat protein removes the IL-7 receptor alpha-chain from the surface of resting CD8 T cells and targets it for degradation. J Immunol 2010;185(5):2854-66.

(105) Kaech SM, Tan JT, Wherry EJ, Konieczny BT, Surh CD, Ahmed R. Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. Nat Immunol 2003;4(12):1191-8.

(106) Franchimont D, Galon J, Vacchio MS, Fan S, Visconti R, Frucht DM, et al. Positive effects of glucocorticoids on T cell function by up-regulation of IL-7 receptor alpha. J Immunol 2002;168(5):2212-8.

(107) Paiardini M, Cervasi B, Albrecht H, Muthukumar A, Dunham R, Gordon S, et al. Loss of CD127 expression defines an expansion of effector CD8+ T cells in HIV-infected individuals. J Immunol 2005;174(5):2900-9.

(108) Lécuroux C, Girault I, Boutboul F, Urrutia A, Goujard C, Meyer L, et al. ntiretroviral therapy initiation during primary HIV infection enhances both CD127 expression and the proliferative capacity of HIV-specific CD8+ T cells. AIDS 2009;23(13):1649-58.

(109) Zhang SY, Zhang Z, Fu JL, Kang FB, Xu XS, Nie WM, et al. Progressive CD127 down-regulation correlates with increased apoptosis of CD8 T cells during chronic HIV-1 infection. Eur J Immunol 2009;39(5):1425-34.

(110) Kiazyk SA, Fowke KR. Loss of CD127 expression links immune activation and CD4(+) T cell loss in HIV infection. Trends Microbiol 2008;16(12):567-73.

(111) Ghazawi FM. Understanding the mechanisms by which interleukin (IL)-7 donw-regulates expression of the IL-7 receptor alpha-chain (CD127) in human CD8 T cells. PhD Thesis 2013.

(112) Ghazawi FM, Faller EM, Sugden SM, Kakal JA, MacPherson PA. IL-7 downregulates IL-7Ralpha expression in human CD8 T cells by two independent mechanisms. Immunol Cell Biol 2013;91(2):149-58.

(113) Yoshimura A, Naka T, Kubo M. SOCS proteins, cytokine signalling and immune regulation. Nat Rev Immunol 2007;7(6):454-65.

(114) Kamura T, Maenaka K, Kotoshiba S, Matsumoto M, Kohda D, Conaway RC, et al. VHL-box and SOCS-box domains determine binding specificity for Cul2-Rbx1 and Cul5-Rbx2 modules of ubiquitin ligases. Genes Dev 2004;18(24):3055-65.

(115) Kamura T, Sato S, Haque D, Liu L, Kaelin WG Jr, Conaway RC, et al. The Elongin BC complex interacts with the conserved SOCS-box motif present in members of the SOCS, ras, WD-40 repeat, and ankyrin repeat families. Genes Dev 1998;12(24):2872-81.

(116) Kile BT, Schulman BA, Alexander WS, Nicola NA, Martin HM, Hilton DJ. The SOCS box: a tale of destruction and degradation. Trends Biochem Sci 2002;27(5):235-41.

(117) Zhang JG, Farley A, Nicholson SE, Willson TA, Zugaro LM, Simpson RJ, et al. The conserved SOCS box motif in suppressors of cytokine signaling binds to elongins B and C and may couple bound proteins to proteasomal degradation. Proc Natl Acad Sci USA 1999;96(5):2071-6.

(118) Goodwin RG, Friend D, Ziegler SF, Jerzy R, Falk BA, Gimpel S, et al. Cloning of the human and murine interleukin-7 receptors Demonstration of a soluble form and homology to a new receptor superfamily. Cell 1990;60(6):941-51.

(119) McElroy CA, Dohm JA, Walsh ST. Structural and biophysical studies of the human IL-7/IL-7Ralpha complex. Structure 2009;17:54-65.

(120) Bazan JF. Structural design and molecular evolution of a cytokine receptor superfamily. Proc Natl Acad Sci USA 1990;87(18):6934-8.

(121) Walsh ST. A biosensor study indicating that entropy, electrostatics, and receptor glycosylation drive the binding interaction between interleukin-7 and its receptor. Biochemistry 2010;49(40):8766-78.

(122) Tanner JW, Chen W, Young RL, Longmore GD, Shaw AS. The conserved box 1 motif of cytokine receptors is required for association with JAK kinases. The Journal of biological chemistry 1995;270:6523-30.

(123) Foxwell BM, Beadling C, Guschin D, Kerr I, Cantrell D. Interleukin-7 can induce the activation of Jak 1 Jak 3 and STAT 5 proteins in murine T cells. Eur J Immunol 1995;25(11):3041-6.

(124) Thomis DC, Berg LJ. The role of Jak3 in lymphoid development, activation, and signaling. Curr Opin Immunol 1997;9:541-7.

(125) Lin JX, Migone TS, Tsang M, Friedmann M, Weatherbee JA, Zhou L, et al. The role of shared receptor motifs and common Stat proteins in the generation of cytokine pleiotropy and redundancy by IL-2, IL-4, IL-7, IL-13, and IL-15. Immunity 1995;2(4):331-9.

(126) Liu W, Putnam AL, Xu-Yu Z, Szot GL, Lee MR, Zhu S, et al. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. J Exp Med 2006;203(7):1701-11.

(127) Clayberger C, Johnson DE, Froelich CJ, Thompson CB, Newmeyer DD, Anel A, et al. A distinct pathway of cell-mediated apoptosis initiated by granulysin. J Immunol 2001;167:350-6.

(128) Sharfe N, Dadi HK, Roifman CM. JAK3 protein tyrosine kinase mediates interleukin-7-induced activation of phosphatidylinositol-3' kinase. Blood 1995;86:2077-85.

(129) Pallard C, Stegmann AP, van Kleffens T, Smart F, Venkitaraman A, Spits H. Distinct roles of the phosphatidylinositol 3-kinase and STAT5 pathways in IL-7-mediated development of human thymocyte precursors. Immunity 1999;10(5):525-35.

(130) Dadi H, Ke S, Roifman CM. Activation of phosphatidylinositol-3 kinase by ligation of the interleukin-7 receptor is dependent on protein tyrosine kinase activity. Blood 1994;84:1579-86.

(131) Venkitaraman AR, Cowling RJ. Interleukin-7 induces the association of phosphatidylinositol 3-kinase with the alpha chain of the interleukin- 7 receptor. Eur J Immunol 1994;24:2168-74.

(132) Kane LP, Weiss A. The PI-3 kinase/Akt pathway and T cell activation: pleiotropic pathways downstream of PIP3. Immunol Rev 2003;192:7-20.

(133) Swainson L, Kinet S, Mongellaz C, Sourisseau M, Henriques T, Taylor N. IL-7-induced proliferation of recent thymic emigrants requires activation of the PI3K pathway. Blood 2007;109(3):1034-42.

(134) Jiang Q, Li WQ, Aiello FB, Klarmann KD, Keller JR, Durum SK. Retroviral transduction of IL-7Ralpha into IL-7Ralpha-/- bone marrow progenitors: correction of lymphoid deficiency and induction of neutrophilia. Gene Ther 2005;12(24):1761-8.

(135) Henriques CM, Rino J, Nibbs RJ, Graham GJ, Barata JT. IL-7 induces rapid clathrin-mediated internalization and JAK3-dependent degradation of IL-7Ralpha in T cells. Blood 2010;115(16):3269-77.

(136) Wofford JA, Wieman HL, Jacobs SR, Zhao Y, Rathmell JC. IL-7 promotes Glut1 trafficking and glucose uptake via STAT5-mediated activation of Akt to support T-cell survival. Blood 2008;111(4):2101-11.

(137) Crawley JB, Rawlinson L, Lali FV, Page TH, Saklatvala J, Foxwell BM. T cell proliferation in response to interleukins 2 and 7 requires p38MAP kinase activation. The Journal of biological chemistry 1997;272:15023-27.

(138) Page TH, Lali FV, Foxwell BM. Interleukin-7 activates p56lck and p59fyn, two tyrosine kinases associated with the p90 interleukin-7 receptor in primary human T cells. Eur J Immunol 1995;25:2956-60.

(139) Seckinger P, Fougereau M. Activation of src family kinases in human pre-B cells by IL-7. J Immunol 1994;153:97-109.

(140) Wells JA. Binding in the growth hormone receptor complex. Proc Natl Acad Sci USA 1996;93(1):1-6.

(141) McElroy CA, Holland PJ, Zhao P, Lim JM, Wells L, Eisenstein E, et al. Structural reorganization of the interleukin-7 signaling complex. Proc Natl Acad Sci USA 2012;109(7):2503-8.

(142) Wickham J Jr, Walsh ST. Crystallization and preliminary X-ray diffraction of human interleukin-7 bound to unglycosylated and glycosylated forms of its alpha-receptor. Acta Crystallogr Sect F Struct Biol Cryst Commun 2007;63(Pt 10):865-9.

(143) Colle JH, Moreau JL, Fontanet A, Lambotte O, Joussemet M, Jacod S, et al. Regulatory dysfunction of the interleukin-7 receptor in CD4 and CD8 lymphocytes from HIV-infected patients—effects of antiretroviral therapy. J Acquir Immune Defic Syndr 2006;42(3):277-85.

(144) Sasson SC, Zaunders JJ, Zanetti G, King EM, Merlin KM, Smith DE, et al. Increased Plasma Interleukin-7 Level Correlates with Decreased CD127 and Increased CD132 Extracellular Expression on T Cell Subsets in Patients with HIV-1 Infection. J Infect Dis 2006;193(4):505-14.

(145) Mackall CL, Fry TJ, Gress RE. Harnessing the biology of IL-7 for therapeutic application. Nat Rev Immunol. 2011;11(5):330-42.

(146) Jeang KT, Xiao H, Rich EA. Multifaceted activities of the HIV-1 transactivator of transcription, Tat. J Biol Chem 1999;274(41):28837-40.

(147) El Kharroubi A, Piras G, Zensen R, Martin MA. Transcriptional activation of the integrated chromatin-associated human immunodeficiency virus type 1 promoter. Mol Cell Biol 1998;18(5):2535-44.

(148) Pessler F, Cron RQ. Reciprocal regulation of the nuclear factor of activated T cells and HIV-1. Genes Immun 2004;5(3):158-67.

(149) Li W, Li G, Steiner J, Nath A. Role of Tat protein in HIV neuropathogenesis. Neurotox Res 2009;16(3):205-20.

(150) Wong JK, Campbell GR, Spector SA. Differential induction of interleukin-10 in monocytes by HIV-1 Clade B and Clade C Tat proteins. J Biol Chem 2010;285(24):18319-25.

(151) Brady J, Kashanchi F. Tat gets the "green" light on transcription initiation. . Retrovirology 2005;2:69.

(152) Sodroski J, Rosen C, Wong-Staal F, Salahuddin SZ, Popovic M, Arya S, et al. Trans-acting transcriptional regulation of human T-cell leukemia virus type III long terminal repeat. Science 1985;227(4683):171-3.

(153) Kao SY, Calman AF, Luciw PA, Peterlin BM. Anti-termination of transcription within the long terminal repeat of HIV-1 by tat gene product. Nature 1987;330(6147):489-93.

(154) Bannwarth S, Gatignol A. HIV-1 TAR RNA: the target of molecular interactions between the virus and its host. Curr HIV Res 2005;3(1):61-71.

(155) Wei P, Garber ME, Fang SM, Fischer WH, Jones KA. A novel CDK9-associated C-type cyclin interacts directly with HIV-1 Tat and mediates its high-affinity, loop-specific binding to TAR RNA. Cell 1998;92(4):451-62.

(156) Li L, Li HS, Pauza CD, Bukrinsky M, Zhao RY. Roles of HIV-1 auxiliary proteins in viral pathogenesis and host-pathogen interactions. Cell Res 2005;11(12):923-34.

(157) Brès V, Tagami H, Péloponèse JM, Loret E, Jeang KT, Nakatani Y, et al. Differential acetylation of Tat coordinates its interaction with the co-activators cyclin T1 and PCAF. EMBO J 2002;21(24):6811-9.

(158) Ratnasabapathy R, Sheldon M, Johal L, Hernandez N. The HIV- 1 long terminal repeat contains an unusual element that induces the synthesis of short RNAs from various mRNA and snRNA promoters. Genes Dev 1990;12A:2061-74.

(159) Kessler M, Mathews MB. Premature termination and processing of human immunodeficiency virus type 1-promoted transcripts. J Virol 1992;66(7):4488-96.

(160) Zhou Q, Sharp PA. Novel mechanism and factor for regulation by HIV-1 Tat. EMBO J 1995;14(2):321-8.

(161) Marshall NF, Price DH. Control of formation of two distinct classes of RNA polymerase II elongation complexes. Mol Cell Biol 1992;12(5):2078-90.

(162) Millhouse S, Manley JL. The C-Terminal Domain of RNA Polymerase II Functions as a Phosphorylation-Dependent Splicing Activator in a Heterologous Protein. Mol Cell Biol 2005;25(2):533-544.

(163) Shilatifard A, Conaway RC, Conaway JW. The RNA polymeraseII elongation complex. Annu Rev Biochem 2003;72:693-715.

(164) Kim DK, Yamaguchi Y, Wada T, Handa H. The regulation of elongation by eukaryotic RNA polymerase II: a recent view. Mol Cells 2001;11(3):267-74.

(165) Richter S, Ping YH, Rana TM. TAR RNA loop: a scaffold for the assembly of a regulatory switch in HIV replication. Proc Natl Acad Sci USA 2002;99(12):7928-33.

(166) Gold MO, Yang X, Herrmann CH, Rice AP. PITALRE, the catalytic subunit of TAK, is required for human immunodeficiency virus Tat transactivation in vivo. J Virol 1998;72(5):4448-53.

(167) Peng J, Zhu Y, Milton JT, Price DH. Identification of multiple cyclin subunits of human P-TEFb. Genes Dev 1998;12(5):755-62.

(168) Bieniasz PD, Grdina TA, Bogerd HP, Cullen BR. Recruitment of a protein complex containing Tat and cyclin T1 to TAR governs the species specificity of HIV-1 Tat. EMBO J 1998;17(23):7056-65.

(169) Barboric M, Peterlin BM. A new paradigm in eukaryotic biology: HIV Tat and the control of transcriptional elongation. PLoS Biol 2005;3(2):e76.

(170) Garber ME, Mayall TP, Suess EM, Meisenhelder J, Thompson NE, Jones KA. CDK9 autophosphorylation regulates high-affinity binding of the human immunodeficiency virus type 1 tat- P-TEFb complex to TAR RNA. Mol Cell Biol 2000;20(18):6958-69.

(171) Kiernan RE, Vanhulle C, Schiltz L, Adam E, Xiao H, Maudoux F, et al. HIV-1 tat transcriptional activity is regulated by acetylation. EMBO J 1999;18(21):6106-18.

(172) Ott M, Schnölzer M, Garnica J, Fischle W, Emiliani S, Rackwitz HR, et al. Acetylation of the HIV-1 Tat protein by p300 is important for its transcriptional activity. Curr Biol 1999;9(24):1489-92.

(173) Col E, Caron C, Seigneurin-Berny D, Gracia J, Favier A, Khochbin S. The histone acetyltransferase, hGCN5, interacts with and acetylates the HIV transactivator, Tat. J Biol Chem 2001;276(30):28179-84.

(174) Brès V, Kiernan R, Emiliani S, Benkirane M. Tat acetyl-acceptor lysines are important for human immunodeficiency virus type-1 replication. J Biol Chem 2002;277(25):22215-21.

(175) Dorr A, Kiermer V, Pedal A, Rackwitz HR, Henklein P, Schubert U, et al. Transcriptional synergy between Tat and PCAF is dependent on the binding of acetylated Tat to the PCAF bromodomain. EMBO J 2002;21(11):2715-23.

(176) Mujtaba S, He Y, Zeng L, Farooq A, Carlson JE, Ott M, et al. Structural basis of lysine-acetylated HIV-1 Tat recognition by PCAF bromodomain. Mol Cell. 2002 Mar;9(3):575-86. 2002;9(3):575-86.

(177) Kaehlcke K, Dorr A, Hetzer-Egger C, Kiermer V, Henklein P, Schnoelzer M, et al. Acetylation of Tat defines a cyclinT1-independent step in HIV transactivation. Mol Cell 2003;12(1):167-76.

(178) Demarchi F, d'Adda di Fagagna F, Falaschi A, Giacca M. Activation of transcription factor NF-kappaB by the Tat protein of human immunodeficiency virus type 1. J Virol 1996;70(7):4427-37.

(179) Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene 1999;18(49):6853-66.

(180) Hiscott J, Kwon H, Génin P. Hostile takeovers: viral appropriation of the NF-kappaB pathway. J Clin Invest 2001;107(2):143-51.

(181) Fiume G, Vecchio E, De Laurentiis A, Trimboli F, Palmieri C, Pisano A, et al. Human immunodeficiency virus-1 Tat activates NF-κB via physical interaction with IκB-α and p65. Nucleic Acids Res 2012;40(8):3548-62.

(182) Puca A, Fiume G, Palmieri C, Trimboli F, Olimpico F, Scala G, et al. IkappaB-alpha represses the transcriptional activity of the HIV-1 Tat transactivator by promoting its nuclear export. J Biol Chem 2007;282(51):37146-57.

(183) Chan JK, Greene WC. NF-κB/Rel: agonist and antagonist roles in HIV-1 latency. Curr Opin HIV AIDS 2011;6(1):12-8.

(184) Brigati C, Giacca M, Noonan DM, Albini A. HIV Tat, its TARgets and the control of viral gene expression. FEMS Microbiol Lett 2003;220(1):57-65.

(185) Campbell GR, Loret EP. What does the structure-function relationship of the HIV-1 Tat protein teach us about developing an AIDS vaccine? Retrovirology 2009;6(50).

(186) Noonan D, Albini A. From the outside in: extracellular activities of HIV Tat. Adv Pharmacol 2000;48:229-50.

(187) Westendorp MO, Frank R, Ochsenbauer C, Stricker K, Dhein J, Walczak H, et al. Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120. Nature 1995;375(6531):497-500.

(188) Gutheil WG, Subramanyam M, Flentke GR, Sanford DG, Munoz E, Huber BT, et al. Human immunodeficiency virus 1 Tat binds to dipeptidyl aminopeptidase IV (CD26): a possible mechanism for Tat's immunosuppressive activity. Proc Natl Acad Sci USA 1994;91(6594):8.

(189) Liu Y, Jones M, Hingtgen CM, Bu G, Laribee N, Tanzi RE, et al. Uptake of HIV-1 Tat protein mediated by low-density lipoprotein receptor-related protein disrupts the neuronal metabolic balance of the receptor ligands. Nat Med 2000;6:1380-7.

(190) Xiao H, Neuveut C, Tiffany HL, Benkirane M, Rich EA, Murphy PM, et al. Selective CXCR4 antagonism by tat: implications for in vivo expansion of coreceptor use by HIV-1. Proc Natl Acad Sci USA 2000;97:11466-71.

(191) Ghezzi S, Noonan DM, Aluigi MG, Vallanti G, Cota M, Benelli R, et al. Inhibition of CXCR4-dependent HIV-1 infection by extracellular HIV-1 Tat. Biochem Biophys Res Commun 2000;270:992-6.

(192) Tyagi M, Rusnati M, Presta M, Giacca M. Internalization of HIV-1 tat requires cell surface heparan sulfate proteoglycans. J Biol Chem 2001;276:3254–3261. 2001;276:3254-61.

(193) Foucault M, Mayol K, Receveur-Bréchot V, Bussat MC, Klinguer-Hamour C, Verrier B, et al. UV and X-ray structural studies of a 101-residue long Tat protein from a HIV-1 primary isolate and of its mutated, detoxified, vaccine candidate. Proteins 2010;78(6):1441-56.

(194) Huigen MC, Kamp W, Nottet HS. Multiple effects of HIV-1 trans-activator protein on the pathogenesis of HIV-1 infection. Eur J Clin Invest 2004;34(1):57-66.

(195) Matsui M, Warburton RJ, Cogswell PC, Baldwin AS Jr, Frelinger JA. Effects of HIV-1 Tat on expression of HLA class I molecules. J Acquir Immune Defic Syndr Hum Retrovirol 1996;11(3):233-40.

(196) Gallo RC. Tat as one key to HIV-induced immune pathogenesis and Tat (correction of Pat) toxoid as an important component of a vaccine. Proc Natl Acad Sci USA 1999;96(15):8324-6.

(197) Cohen SS, Li C, Ding L, Cao Y, Pardee AB, Shevach EM, et al. Pronounced acute immunosuppression in vivo mediated by HIV Tat challenge. Proc Natl Acad Sci USA. 1999;96(19):10842-7.

(198) Zagury D, Lachgar A, Chams V, Fall LS, Bernard J, Zagury JF, et al. Interferon alpha and Tat involvement in the immunosuppression of uninfected T cells and C-C chemokine decline in AIDS. Proc Natl Acad Sci USA 1998;95(7):3851-6.

(199) Benjouad A, Mabrouk K, Moulard M, Gluckman JC, Rochat H, Van Rietschoten J, et al. Cytotoxic effect on lymphocytes of Tat from human immunodeficiency virus (HIV-1). FEBS Lett 1993;319(1-2):119-24.

(200) Viscidi RP, Mayur K, Lederman HM, Frankel AD. Inhibition of antigen-induced lymphocyte proliferation by Tat protein from HIV-1. Science 1989;246(4937):1606-8.

(201) Zocchi MR, Rubartelli A, Morgavi P, Poggi A. HIV-1 Tat inhibits human natural killer cell function by blocking 1-type calcium channels. J Immunol 1998;161(6):2938-43.

(202) Verhoef K, Bauer M, Meyerhans A, Berkhout B. On the role of the second coding exon of the HIV-1 Tat protein in virus replication and MHC class I downregulation. AIDS Res Hum Retroviruses 1998;15(17):1553-9.

(203) Barton CH, Biggs TE, Mee TR, Mann DA. The human immunodeficiency virus type 1 regulatory protein Tat inhibits interferon-induced iNos activity in a murine macrophage cell line. J Gen Virol 1996;77(Pt8):1643-7.

(204) Huang L, Bosch I, Hofmann W, Sodroski J, Pardee AB. Tat protein induces human immunodeficiency virus type 1 (HIV-1) coreceptors and promotes infection with both macrophage-tropic and T-lymphotropic HIV-1 strains. J Virol 1998;72:8952-60.

(205) Secchiero P, Zella D, Capitani S, Gallo RC, Zauli G. Extracellular HIV-1 tat protein up-regulates the expression of surface CXC-chemokine receptor 4 in resting CD4+ T cells. J Immunol 1999;162:2427-31.

(206) Albini A, Barillari G, Benelli R, Gallo RC, Ensoli B. Angiogenic properties of human immunodeficiency virus type 1 Tat protein. Proc Natl Acad Sci USA 1995;92(11):4838-42.

(207) Benelli R, Barbero A, Ferrini S, Scapini P, Cassatella M, Bussolino F, et al. Human immunodeficiency virus transactivator protein (Tat) stimulates chemotaxis, calcium mobilization, and activation of human polymorphonuclear leukocytes: implications for Tat-mediated pathogenesis. J Infect Dis 2000;182(6):1643-51.

(208) Albini A, Ferrini S, Benelli R, Sforzini S, Giunciuglio D, Aluigi MG, et al. HIV-1 Tat protein mimicry of chemokines. Proc Natl Acad Sci USA 1998;95(22):13153-8.

(209) Benelli R, Mortarini R, Anichini A, Giunciuglio D, Noonan DM, Montalti S, et al. Monocyte-derived dendritic cells and monocytes migrate to HIV-Tat RGD and basic peptides. AIDS 1998;12(3):261-8.

(210) Jones KA, Peterlin BM. Control of RNA initiation and elongation at the HIV-1 promoter. Annu Rev Biochem 1994;63:717-43.

(211) Ruben S, Perkins A, Purcell R, Joung K, Sia R, Burghoff R, et al. Structural and functional characterization of human immunodeficiency virus tat protein. J Virol 1989;63(1):1-8.

(212) Malim MH, Hauber J, Fenrick R, Cullen BR. Immunodeficiency virus rev trans-activator modulates the expression of the viral regulatory genes. Nature 1988;335(6186):181-3.

(213) Rayne F, Debaisieux S, Yezid H, Lin YL, Mettling C, Konate K, et al. Phosphatidylinositol-(4,5)-bisphosphate enables efficient secretion of HIV-1 Tat by infected T-cells. EMBO J 2010;29(8):1348-62.

(214) Yezid H, Konate K, Debaisieux S, Bonhoure A, Beaumelle B. Mechanism for HIV-1 Tat insertion into the endosome membrane. J Biol Chem 2009;284(34):22736-46.

(215) Garcia JA, Harrich D, Pearson L, Mitsuyasu R, Gaynor RB. Functional domains required for tat-induced transcriptional activation of the HIV-1 long terminal repeat. EMBO J 1988;7(10):3143-7.

(216) Tahirov TH, Babayeva ND, Varzavand K, Cooper JJ, Sedore SC, Price DH. Crystal structure of HIV-1 Tat complexed with human P-TEFb. Nature 2010;465(7299):747-51.

(217) Churcher MJ, Lamont C, Hamy F, Dingwall C, Green SM, Lowe AD, et al. High affinity binding of TAR RNA by the human immunodeficiency virus type-1 tat protein requires base-pairs in the RNA stem and amino acid residues flanking the basic region. J Mol Biol 1993;230(1):90-110.

(218) Hauber J, Malim MH, Cullen BR. Mutational analysis of the conserved basic domain of human immunodeficiency virus tat protein. J Virol 1989;63(3):1181-7.

(219) de Mareuil J, Carre M, Barbier P, Campbell GR, Lancelot S, Opi S, et al. HIV-1 Tat protein enhances microtubule polymerization. Retrovirology. 2005 Feb 3;2:5. 2005;2(5).

(220) Barillari G, Gendelman R, Gallo RC, Ensoli B. The Tat protein of human immunodeficiency virus type 1, a growth factor for AIDS Kaposi sarcoma and cytokine-activated vascular cells, induces adhesion of the same cell types by using integrin receptors recognizing the RGD amino acid sequence. Proc Natl Acad Sci USA 1993;90(17):7941-5.

(221) Mahlknecht U, Dichamp I, Varin A, Van Lint C, Herbein G. NF-kappaB-dependent control of HIV-1 transcription by the second coding exon of Tat in T cells. J Leukoc Biol 2008;83(3):718-27.

(222) López-Huertas MR, Callejas S, Abia D, Mateos E, Dopazo A, Alcamí J, et al. Modifications in host cell cytoskeleton structure and function mediated by intracellular HIV-1 Tat protein are greatly dependent on the second coding exon. Nucleic Acids Res 2010;38(10):3287-307.

(223) Faller EM, McVey MJ, Kakal JA, MacPherson PA. Interleukin-7 receptor expression on CD8 T-cells is downregulated by the HIV Tat protein. J Acquir Immune Defic Syndr 2006;43(3):257-69.

(224) Faller E, Kakal J, Kumar R, Macpherson P. IL-7 and the HIV Tat protein act synergistically to down-regulate CD127 expression on CD8 T cells. Int Immunol 2009;21(3):203-16.

(225) Sugden SM. Mutational Analysis of the HIV-1 Tat Protein and Its Role in Downregulating CD127 on CD8 T cells. PhD Thesis 2013.

(226) OriGene®. pCMV6-CD127 plasmid. Available at: URL: http://www.origene.com/orf_clone/trueclone/NM_002185/RC209687/IL7R.aspx. Accessed 4/24, 2014.

(227) Agilent Technologies ©. QuikChange II XL Site-Directed Mutagenesis Kit Instruction Manual. Available at: http://www.chem.agilent.com/library/usermanuals/Public/200521.pdf. Accessed 4/24/2014.

(228) Gee K, Angel JB, Mishra S, Blahoianu MA, Kumar A. IL-10 regulation by HIV-Tat in primary human monocytic cells: involvement of calmodulin/calmodulin-dependent protein kinase-activated p38 MAPK and Sp-1 and CREB-1 transcription factors. J Immunol 2012;188(3553).

(229) Brand SR, Kobayashi R, Mathews MB. The Tat protein of human immunodeficiency virus type 1 is a substrate and inhibitor of the interferon-induced, virally activated protein kinase, PKR. J Biol Chem, 1997, Vol. 272, pp. 8388-95. 1997;272(13):8388-95.

(230) Leghmari K, Contreras X, Moureau C, Bahraoui E. HIV-1 Tat protein induces TNF-alpha and IL-10 production by human macrophages: differential implication of PKC-betaII and -delta isozymes and MAP kinases ERK1/2 and p38. Cell Immunol 2008;254(1):46-55.

(231) Alves NL, van Leeuwen EM, Derks IA, van Lier RA. Differential regulation of human IL-7 receptor alpha expression by IL-7 and TCR signaling. J Immunol, 2008;180(8):5201-10.

(232) Kim HR, Hwang KA, Kim KC, Kang I. *Down-regulation of IL-7Ralpha expression in human T cells via DNA methylation.* s.l. : J. Immunol. 2007;178:5473-79.

(233) Akhtar LN, Benveniste EN. Viral exploitation of host SOCS protein functions. J Virol. 2011;85(5):1912-21.

(234) Rezza G, Fiorelli V, Dorrucci M, Ciccozzi M, Tripiciano A, Scoglio A, et al. The presence of anti-Tat antibodies is predictive of long-term nonprogression to AIDS or severe immunodeficiency: findings in a cohort of HIV-1 seroconverters. J Infect Dis. 2005;191(8):1321-4.

(235) Richardson MW, Mirchandani J, Duong J, Grimaldo S, Kocieda V, Hendel H, et al. Antibodies to Tat and Vpr in the GRIV cohort: differential association with maintenance of long-term non-progression status in HIV-1 infection. Biomed Pharmacother. 2003;57(1):4-14.

(236) van Baalen CA, Pontesilli O, Huisman RC, Geretti AM, Klein MR, de Wolf F, et al. Human immunodeficiency virus type 1 Rev- and Tat-specific cytotoxic T lymphocyte frequencies inversely correlate with rapid progression to AIDS. J Gen Virol. 1997;78(Pt 8):1913-8.

(237) Addo MM, Altfeld M, Rosenberg ES, Eldridge RL, Philips MN, Habeeb K, et al. The HIV-1 regulatory proteins Tat and Rev are frequently targeted by cytotoxic T lymphocytes derived from HIV-1-infected individuals. Proc Natl Acad Sci USA. 2001;98(4):1781-6.

(238) Cao J, McNevin J, Holte S, Fink L, Corey L, McElrath MJ. Comprehensive analysis of human immunodeficiency virus type 1 (HIV-1)-specific gamma interferon-secreting CD8+ T cells in primary HIV-1 infection. J Virol. 2003;77(12):6867-78.

(239) Wei SJ, Metz JM, Coyle C, Hampshire M, Jones HA, Markowitz S, et al. Recruitment of patients into an internet-based clinical trials database: the experience of OncoLink and the National Colorectal Cancer Research Alliance. J Clin Oncol. 2004;22(23):4730-6.

(240) Allen TM, O'Connor DH, Jing P, Dzuris JL, Mothé BR, Vogel TU, et al. Tat-specific cytotoxic T lymphocytes select for SIV escape variants during resolution of primary viraemia. Nature. 2000;407(6802):386-90.

(241) Ensoli B, Fiorelli V, Ensoli F, Lazzarin A, Visintini R, Narciso P, et al. The therapeutic phase I trial of the recombinant native HIV-1 Tat protein. AIDS. 2008;22(16):2207-9.

(242) Ensoli B, Fiorelli V, Ensoli F, Lazzarin A, Visintini R, Narciso P, et al. The preventive phase I trial with the HIV-1 Tat-based vaccine. Vaccine. 2009;28(2):371-8.

(243) Bellino S, Francavilla V, Longo O, Tripiciano A, Paniccia G, Arancio A, et al. Parallel conduction of the phase I preventive and therapeutic trials based on the Tat vaccine candidate. Rev Recent Clin Trials. 2009;4(3):195-204.

(244) Longo O, Tripiciano A, Fiorelli V, Bellino S, Scoglio A, Collacchi B, et al. Phase I therapeutic trial of the HIV-1 Tat protein and long term follow-up. Vaccine. 2009;27(25-26):3306-12.

(245) Maggiorella MT, Baroncelli S, Michelini Z, Fanales-Belasio E, Moretti S, Sernicola L, et al. Long-term protection against SHIV89.6P replication in HIV-1 Tat vaccinated cynomolgus monkeys. Vaccine. 2004;22(25-26):3258-69.

(246) Cafaro A, Caputo A, Fracasso C, Maggiorella MT, Goletti D, Baroncelli S, et al. Control of SHIV-89.6P-infection of cynomolgus monkeys by HIV-1 Tat protein vaccine. Nat Med. 1999;5(6):643-50.

(247) Fanales-Belasio E, Moretti S, Fiorelli V, Tripiciano A, Pavone Cossut MR, Scoglio A, et al. HIV-1 Tat addresses dendritic cells to induce a predominant Th1-type adaptive immune response that appears prevalent in the asymptomatic stage of infection. J Immunol. 2009;182(5):2888-97.

(248) Fanales-Belasio E, Moretti S, Nappi F, Barillari G, Micheletti F, Cafaro A, et al. Native HIV-1 Tat protein targets monocyte-derived dendritic cells and enhances their maturation, function, and antigen-specific T cell responses. J Immunol. 2002;168(1):197-206.

(249) Chang HC, Samaniego F, Nair BC, Buonaguro L, Ensoli B. HIV-1 Tat protein exits from cells via a leaderless secretory pathway and binds to extracellular matrix-associated heparan sulfate proteoglycans through its basic region. AIDS. 1997;11(12):1421-31.

(250) Ensoli B, Bellino S, Tripiciano A, Longo O, Francavilla V, Marcotullio S, et al. Therapeutic Immunization with HIV-1 Tat Reduces Immune Activation and Loss of Regulatory T-Cells and Improves Immune Function in Subjects on HAART. PLoS One. 2010;5(11):e13540.

(251) Appay V, Sauce D. Immune activation and inflammation in HIV-1 infection: causes and consequences. J Pathol. 2008;214(2):231-41.

(252) Deeks SG, Phillips AN. HIV infection, antiretroviral treatment, ageing, and non-AIDS related morbidity. BMJ. 2009;338:a3172.

(253) Robbins GK, Spritzler JG, Chan ES, Asmuth DM, Gandhi RT, Rodriguez BA, et al. Incomplete reconstitution of T cell subsets on combination antiretroviral therapy in the AIDS Clinical Trials Group protocol 384. Clin Infect Dis. 2009;48(3):350-61.

(254) Walensky RP, Paltiel AD, Losina E, Mercincavage LM, Schackman BR, Sax PE, et al. The survival benefits of AIDS treatment in the United States. J Infect Dis. 2006;194(1):11-9.

7. Appendix

7.1 Image Reproduction Permissions

Figure 1: Structure of the HIV-1 virion. As well as **Figure 2:** The HIV-1 genome.

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Figure 3: A schematic representation of the HIV-1 promoter which is located within the 5' LTR of the viral genome. As well as **Figure 11**. TAR RNA Structure. Tat recognition primarily requires interactions with the bulge region of TAR.

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Figure 4: The HIV-1 cellular life cycle.

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Figure 6. Cumulative model illustrating homeostatic recycling of CD127 at the cell surface and down regulation of CD127 surface protein and gene transcription following IL-7 stimulation.

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Yes absolutely you have my permission to use the figure from my thesis (Figure 49: Cumulative model illustrating homeostatic recycling of CD127 at the cell surface and down regulation of CD127 surface protein and gene transcription following IL-7 stimulation) with referencing.

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Feras Al-Ghazawi

Figure 8. Interleukin-7 receptor signalling cascades. IL-7 signals through the IL-7 receptor, a heterodimer comprised of CD127 and CD132.

Figure 10: HIV-1 *Trans*-activator protein is composed of six regions.

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Figure 11. pCMV6-CD127 plasmid back bone, containing the CD127 cDNA cloned downstream of the CMV promoter from OriGene®.

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Case Update: TC11262261 - Permission to use pCMV6-Entry backbone in my thesis

2 messages

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Thank you very much for your email. Yes, you can certainly use the diagram in your thesis. All you need is to put the correct citation in your thesis.

Best,

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Here is some information about it:
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Thanks,
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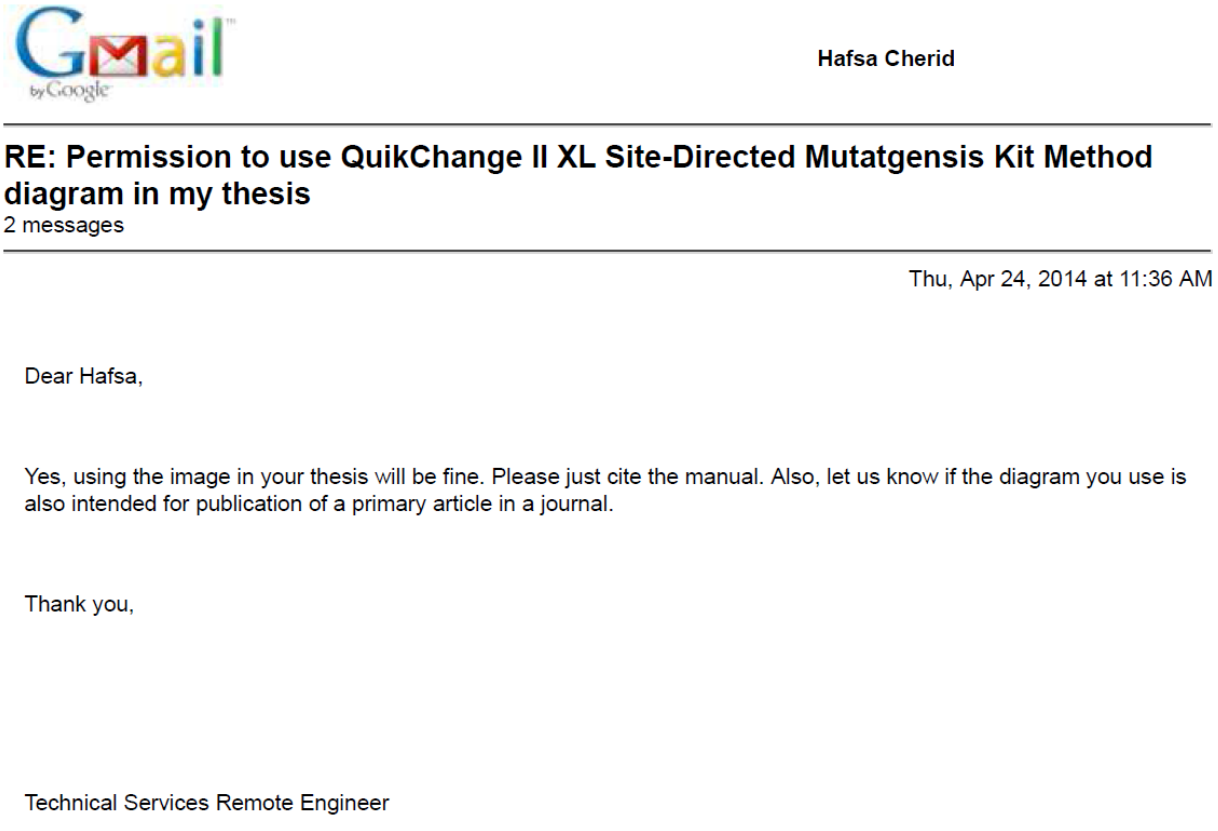
Figure 13. Overview of the QuickChange® II XL-site directed mutagenesis method.



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7.2 Ethical approval by the Ottawa Hospital Research Institute and blood consent forms

The Ottawa Hospital | L'Hôpital d'Ottawa

Information Sheet and Consent Form for Blood and/or Semen Collection and/or Colon Biopsies

Study: Impaired Immune Function during HIV Infection

Principal Investigator: Dr. Paul MacPherson

Introduction:
You are being asked to participate in a study focused on immune function in HIV infection. Before you decide to participate, it is important that you understand why this research is being conducted. Please take a moment to read the following information, and ask questions if there is anything that you find unclear.

Background:
HIV infection over time causes severe impairment of the immune system. HIV enters some immune cells such as CD4 T-cells and monocytes and either kills them or prevents them from functioning properly. Other immune cells such as CD8 T-cells are not infected by HIV but are still unable to function in the presence of HIV. By limiting the activity of these various immune cells, HIV weakens the entire immune system. When the immune system is weak, HIV is able to replicate freely and eventually people can become sick from opportunistic infections.
How HIV inactivates these different cells is not yet understood.

HIV infected cells travel through the blood to many sites in the body including the testicles and prostate. Once in these tissues, HIV may behave differently than when it is present in the blood or lymph nodes. Sometimes HIV can be detected in the semen even when it is undetectable in the blood. Control of HIV replication in the semen then may not perfectly mirror what occurs in the blood.

HIV can also infect the cells that line the colon, a part of the digestive tract, and the effect it has on the immune system in the gut may also not mirror what occurs in the blood or semen.

Purpose:
One of the goals of this research program is to determine how the activities of various immune cells change during HIV infection. This will be done by examining changes in the expression of molecules on the cell surface as well as changes in overall cell function. We will compare these changes between HIV negative individuals, HIV+ patients with active viral replication, HIV+ patients on antiretroviral therapy, and in long term nonprogressors.

Another goal of this research program is to determine how immunologic control of HIV differs between different tissue compartments, namely the blood, semen, and the digestive tract. This will be done by examining HIV viral loads and levels of immune modulators isolated from semen

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or gut. Cells of the immune system involved in controlling HIV replication may also be isolated from semen or gut in order to examine their functional abilities.

Study Procedures:
Participants will be typically asked to provide a sample of either blood **or** semen **or** gut tissue. Participants who provide semen or gut samples may be also asked to provide blood.

Blood Samples:
Participants will be asked to donate samples of blood ranging from 10 ml, less than 1 tablespoon ~~(one tube) to a maximum of 200ml, approximately 14 tablespoon (less than one half the volume~~ drawn by Canadian Blood Services for routine blood donation; one unit = 500ml, approximately 33 tablespoon). White cells will be isolated from the blood and analyzed in the laboratory for changes in the expression of different molecules, and for immune activity. Serum may also be examined for molecules which regulate the activity of the immune cells.

Semen Samples:
Participants may be asked to donate semen samples. Sterile containers will be provided. Samples will be collected privately by the participant but must be delivered to the clinic within two hours.

Gut Samples:
During your colonoscopy procedure small biopsies of the colon wall will be performed in order to gather gut-associated lymphoid tissue. Approximately 30 tissue biopsies will be obtained, each consisting of about 0.3mm of tissue (9 mm total).

Duration:
Your participation will be complete once the blood and/or semen and/or gut tissue sample is obtained.

Risks:

Blood Samples:
The risks of participating in this study are those associated with routine blood drawing and may include minor pain at the site where blood is drawn and minimal bruising. Some people may experience temporary lightheadedness after drawing blood. If this occurs, you may be asked to rest in the clinic for a short period of time until the lightheadedness passes.

Semen Samples:
There are no risks associated with semen donation. Semen samples will only be used to determine HIV viral loads and for immunologic studies. Under no circumstances will the semen be available to achieve fertilization under any conditions.

Gut Samples:
During the procedure, you may feel pressure in the rectum similar to the sensation you feel with the urge to have a bowel movement. You may also feel a small amount of cramping in your abdomen as well. The most serious risk of the procedure is perforation, poking a hole in the

lining of the intestine, but it is extremely uncommon, occurring once out of every 10,000 procedures. If this occurs the attending physician will be responsible for treatment. Some bleeding will likely occur at the point where the biopsies were done. If you should experience severe discomfort during colon mucosa biopsy the procedure will be stopped immediately; you will be seen by the physician and the research nurse will monitor you until you are ready to go home.

Due to the possible bleeding following the procedure, the risk of transmitting HIV after the biopsy procedure may be increased.

Benefits:

Participation in this study will provide no direct benefits to you. It will, however, help to advance our understanding of how HIV inactivates the immune system and may lead to new strategies for the development of immune based therapies to control HIV replication.

Voluntary Participation and Withdrawal:

You are under no obligation to participate in this study. If you choose to participate, you may change your mind at any time without providing a reason. You should inform the study doctor/study staff if you decide to withdraw so that your sample may be destroyed. You are not waiving your legal rights by agreeing to take part. Whether you choose to participate or not will have absolutely no effect on the medical care you receive, now or in the future.

Confidentiality:

All personal health information will be kept confidential, unless release is required by law. Representatives of the Ottawa Hospital Research Ethics Board, as well as the Ottawa Hospital Research Institute, may review your original medical records under the supervision of Dr. MacPherson's staff for audit purposes.

You will not be identifiable in any publications or presentations resulting from this study. No identifying information will leave the Ottawa Hospital.

The link between your name and the independent study number will only be accessible by Dr. MacPherson and/or his staff. The link and study files will be stored separately and securely. Both files will be kept for a period of 15 years after the study has been completed. All paper records will be stored in a locked file and/or office. All electronic records will be stored and protected by a user password, again only accessible by Dr. MacPherson and/or his staff. At the end of the retention period, all paper records will be disposed of in confidential waste or shredded, and all electronic records will be deleted.

Your chart will be used only to obtain CD4 counts, viral loads, and overall health status. Data will be compiled using a code. Your name will not be used and will not be known to laboratory personnel. The results of this study may be presented at meetings or in publications. Your identity will not be disclosed at any time in any of these presentations or publications.

Questions About The Study:

If during the course of this study you have questions concerning the study, you may contact the principle investigator Dr Paul MacPherson

The Ottawa Hospital Research Ethics Board has approved this study. This committee considers the ethical aspects of all research projects involving people. If you have any questions about your rights with regard to participating in a research study, you may contact the Chair of the Ottawa Hospital Research Ethics Board at 613-798-5555, extension 14902. Do not sign the consent form unless you have had a chance to ask questions and have received satisfactory answers to all you questions. By signing the consent form you are not waiving your legal rights.

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Consent Form
Impaired Immune Function during HIV Infection

Consent to Participate in Research

I understand that I am being asked to participate in a research study about immune function in HIV infection. This study has been explained to me.

I have read this, 5-page Participant Information Sheet and Consent Form (or have had this document read to me). All my questions have been answered to my satisfaction. If I decide at a later stage in the study that I would like to withdraw my consent, I may do so at any time.

I voluntarily agree to participate in this study and will provide a:

Please Initial:

_____Blood Sample

_____Semen Sample

_____Gut Sample

A copy of the signed Information Sheet and/or Consent Form will be provided to me.

Signatures

_____
Participant's Name (Please Print)

_____ _____
Participant's Signature Date

Investigator Statement (or Person Explaining the Consent)

I have carefully explained to the research participant the nature of the above research study. To the best of my knowledge, the research participant signing this consent form understands the nature, demands, risks and benefits involved in participating in this study. I acknowledge my responsibility for the care and well being of the above research participant, to respect the rights and wishes of the research participant, and to conduct the study according to applicable Good Clinical Practice guidelines and regulations.

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8. Contributions of Collaborators

I would like to acknowledge collaborators and also thank everyone who gave me advice. I particularly acknowledge Dr. Feras Al Ghazawi: carried out work in figs 19A and 20A.

9. Curriculum Vitae

Hafsa Cherid

Education

Master's of Science: Microbiology and Immunology	University of Ottawa	05/2010 – current
Bachelors of Science, Honors: Integrated Science, minor chemistry	Carleton University	09/2005 – 04/2009

Academic Distinctions

2013	Ontario Graduate Scholarship in Science and Technology .
2012	University of Ottawa Excellence Scholarship.
2012	Research Trainee Award
2012	Ontario Graduate Scholarship in Science and Technology.
2010	University of Ottawa Excellence Scholarship.
2010	Ontario Graduate Scholarship in Science and Technology.
2009	University of Ottawa Dean's Honour's List.
2009	Undergraduate Student Research Award (USRA)
2008	Carleton University Merit Scholarship for Outstanding Academic Achievement.
2008	Rising Stars of Research 2008 Research Conference Travel Grant.
2008	University of Ottawa Dean's Honour's List.
2008	Undergraduate Student Research Award (USRA)
2007	Carleton University Merit Scholarship for Outstanding Academic Achievement.
2007	University of Ottawa Dean's Honour's List.
2007	Undergraduate Student Research Award (USRA)
2006	Carleton University Merit Scholarship for Outstanding Academic Achievement.
2006	University of Ottawa Dean's Honour's List.
2005	Carleton University Merit Scholarship for Outstanding Academic Achievement.
2005	Principle's Commendation Award
2005	Ontario scholar award
2004	Explore Program Bursary – French studies

Research Experience

05/2010 – current	*Regions of the CD127 cytoplasmic domain necessary for Tat binding.* **P.I. Dr. Paul MacPherson**. University of Ottawa, Department of Biochemistry, Microbiology and Immunology.
08/2008 – 08/2009	*Performance of Two Different Limulus Amebocyte Lysate Assays for the Quantitation of Fungal Gluca.*

	P.I. Dr. J. David Miller. Carleton University, Department of Biochemistry.
05/2005 – 08/2005	*Horizontal transmission of the Picea glauca foliar endophyte Phialocephala scopiformis CBS 120377.* **P.I. Dr. J. David Miller**. Carleton University, Department of Biochemistry.
05/2006 – 08/2006	*Horizontal transmission of the Picea glauca foliar endophyte Phialocephala scopiformis CBS 120377.* **P.I. Dr. J. David Miller.** Carleton University, Department of Biochemistry.
05/2007 – 08/2007	*Horizontal transmission of the Picea glauca foliar endophyte Phialocephala scopiformis CBS 120377.* **P.I. Dr. J. David Miller.** Carleton University, Department of Biochemistry.

Leadership Roles--Trainees Under Direct Tutelage

Honour's Student	3

Other Teaching Experience and Tutoring

2009 – 2010	Carleton University Teaching Assistant: Chemistry Department
2009 – 2010	Ottawa Islamic School Grade 12 Biology Teacher

Knowledge Transfer and Community Outreach Activities

2009	HIV Medical Project with Fondation Citoyens du Monde (FOCIMO) : Goudévé, Togo,Africa
2009 – currnet	Canadian Red Cross first aider
2009 – 2010	Monfort Hospital patient care giver
2008 – 2009	Let's Talk Science Program and Science Travels presenter/ educator
2008 – current	Canadian Red Cross personal disaster assistant
2007 – 2009	MS Society Bike Tour, Terry Fox Run, Snow Suit Fund volunteer

Published Abstracts

1. Feras Ghazawi, Elliott Faller, Parmvir Parmar, Scott Sugden, Juzer Kakal, **Hafsa Cherid** and Paul MacPherson. (2013). IL-7 suppresses CD127 gene transcription in human CD8 T cells by inducing expression of the transcription factor c-Myb (poster presentation). *100th Annual Meeting - The American Association of Immunologists. Honolulu, Hawaii, USA*
2. **Hafsa Cherid**, Feras Ghazawi and Paul MacPherson. (2013). Regions of the CD127 cytoplasmic domain necessary for Tat-induced internalization (poster presentation). *22nd Annual Canadian Conference on HIV/AIDS Research. Vancouver, British Columbia, Canada*
3. Paul MacPherson, Feras Ghazawi, Elliott Faller, Scott Sugden, **Hafsa Cherid**, Juzer Kakal, Parmvir Parmar and Abdulkareem El-Salfiti. (2013). By usurping IL-7 mediated pathways, the HIV Tat protein targets CD127 for degradation and thus attenuates CD8 T-cell survival and

function (oral presentation). *22nd Annual Canadian Conference on HIV/AIDS Research. Vancouver, British Columbia, Canada*

4. Feras Ghazawi, Parmvir Parmar, Elliott Faller, Scott Sugden, **Hafsa Cherid**, Juzer Kakal, Abdulkareem El Salfiti and Paul MacPherson. (2013). Understanding the Mechanisms by which IL-7 Down-regulates Expression of the IL-7 Receptor Alpha-chain (CD127) in Primary Human CD8 T-Cells (poster presentation). *20th conference on Retroviruses and Opportunistic Infections (CROI). Atlanta, Georgia, USA*
5. **Hafsa Cherid** and David J. Miller. (2008). Performance of Two Different Limulus Amebocyte Lysate Assays for the quantitation of Fungal Gluca. *Rising Stars of Research. Vancouver, British Columbia, Canada*

Conference Proceedings/Abstracts Published in Journals

1. Feras Ghazawi, Elliott Faller, Parmvir Parmar, Scott Sugden, Juzer Kakal, **Hafsa Cherid** and Paul MacPherson. (2013). IL-7 suppresses CD127 gene transcription in human CD8 T cells by inducing expression of the transcription factor c-Myb. The Journal of Immunology, May 2013, Volume 190. Meeting Abstract Supplement 184.11.
2. **Hafsa Cherid**, Feras Ghazawi and Paul MacPherson. (2013). Regions of the CD127 cytoplasmic domain necessary for Tat-induced internalization. The Canadian Journal of Infectious Diseases and Medical Microbiology. Spring 2013, Volume 24. Supplement SB. Abstract P001.
3. Paul MacPherson, Feras Ghazawi, Elliott Faller, Scott Sugden, **Hafsa Cherid**, Juzer Kakal, Parmvir Parmar and Abdulkareem El-Salfiti. (2013). By usurping IL-7 mediated pathways, the HIV Tat protein targets CD127 for degradation and thus attenuates CD8 T-cell survival and function. The Canadian Journal of Infectious Diseases and Medical Microbiology. Spring 2013, Volume 24. Supplement SB. Abstract O025.

Manuscripts

1. **Hafsa Cherid**, Mark Foto, J. David Miller. Performance of Two Different Limulus Amebocyte Lysate Assays for the Quantitation of Fungal Glucan. J Occup Environ Hyg. 2011. 8(9): 540 – 3.

2. J. David Miller, **Hafsa Cherid**, Mark W. Sumarah, Gregory W. Adams. Horizontal transmission of the *Picea glauca* foliar endophyte *Phialocephala scopiformis* CBS 120377. Fungal Ecology. 2009. 2(2): 98 – 101.