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Assessment of HIV infection in cells of infected individuals

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Abstract. HIV is one of the most prevalent opportunistic pathogens in cells of infected individuals and can cause diseases such as: encephalitis, pneumonia and chorioretinitis. The aim of this study is to investigate HIV infection in cells infected persons. This study was performed on 50 blood samples from individuals infected with HIV. Studied patients in the two groups were consumer and nonconsumer of antiretroviral drugs (mention the name of the Antiviral drug). After separation of plasma from the blood samples to detect HIV in cell culture and PCR were examined. Of the 50 patients, 56 % (n = 28) were male and 44 percent (n = 22) were women. DNA antibodies in 16 % (8 cases) were identified in the population, the prevalence of HIV infection in men 21.4 % (6 cases) and in women 1.9% (2 cases). The high prevalence of HIV infection in patients treated with CMV (full form of CMV), the treatment of HIV infection should be considered.
Keyword: HIV infection, cells infection, analyses, characteristics.

Introduction

The precise understanding of the molecular mechanisms in each step of the human immunodeficiency virus (HIV) life cycle has provided an essential basis for designing antiviral compounds and strategies aimed at blocking viral replication and preventing or delaying disease progression [MELLORS *et al.*, 1996].

As in other retroviral infections, the replication cycle of HIV can be described as proceeding in two phases.

The first phase includes entry of the virion into the cell cytoplasm, synthesis of double–stranded DNA (provirus) using the single–stranded genome RNA as a template, transfer of the proviral DNA to the nucleus, and integration of the DNA into the host genome [KINTER *et al.*, 2004].

The second phase includes synthesis of new copies of the viral genome, expression of viral genes, virion assembly by encapsidation of the genome by precursors of the HIV structural proteins, budding, and processing of the viral proteins. Whereas the former phase is mediated by proteins that are present within the virion and occurs in the absence of viral gene expression, the latter, leading to production of infectious virions, is a complex process requiring the interplay of viral and cellular factors [MACATONIA *et al.*, 1990].

The precise understanding of the molecular mechanisms of HIV replication and the use of new technologies in virology has led to exciting discoveries on new aspects of the biology of this virus.

In particular, a growing body of new data on the HIV replication mechanisms together with the results from molec–3 *Cellular Aspects of HIV Infection* [LI *et al.*, 2005]. Edited by Andrea Cossarizza, David Kaplanular studies carried out directly in vivo have allowed researchers to address the virus–host relationships, including the pathogenic role of this virus in disease progression.

In human immunodeficiency virus (HIV) disease, as in all viral infections, CD8 T–cells constitute a critical component of the protective immune response. Loss of CD8 T–cell activity coincides with the progression to acquired immunodeficiency syndrome (AIDS), and studies on long–term nonprogressors have underscored the importance of cytotoxic T lymphocyte (CTL) function

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[FINZI *et al.*, 1999]. One of the intriguing alterations in the peripheral T–cell pool of individuals infected with HIV is the progressive accumulation within the CD8 T–cell subset of a population of cells that lack expression of the CD28 costimulatory molecule [ADDO *et al.*, 2003].

Indeed, in some HIV–infected persons, >65 % of the CD8 T cells are CD28. A more complete characterization of this unusual cell population, therefore, is essential for understanding disease pathogenesis as well as for the development of appropriate strategies for treatment [CONNOR *et al.*, 1997].

Because CD28 T cells are poorly proliferative, they do not contribute to production of soluble antiviral suppressive factors, and also show alterations in apoptosis and in cell–cell adhesion.

The presence of large proportions of such cells will undoubtedly have a profound in influence on the immune control over HIV infection.

Although there had been much speculation by AIDS researchers on the origin of the CD28 T cells, elucidation of the nature of this expanded population of cells in HIV disease has emerged from research in a totally different arena of scientific investigation, namely basic cell biology studies on the process of replicative senescence [DONNELL *et al.*, 2010].

Human immunodeficiency virus (HIV) infection is associated with a complex pattern of alterations that profoundly, the immune response [CALAROTA *et al.*,1998].

HIV–induced immune dysregulation impairs both the quantitative and qualitative homeostasis of the immune system, and this impairment is manifested as an array of multiple, characteristic defects that can be summarized as follows: A progressive decline in CD4 T–lymphocyte numbers and a profound impairment of the functionality of these cells.

A decrease in interleukin IL–2, IL–12, IL–15, and interferon (IFN) g (type–1 cytokines that mainly stimulate cell–mediated immunity and immune defences against intracellular pathogens) and an increase in IL–4, IL–5, IL–6, and IL–10 production (type–2 cytokines that mainly stimulate antibody production and humoral immunity and are analysed at the activation of immune defences against extracellular pathogens).

Changes in immunophenotype of HIV–infected cells ± CD4 are shown (Table 1). T cells increase in the subpopulations expressing CD45RO, Fas.

Table 1.

A Brief Summary of the Main Changes Observed When Immunophenotyping Is Performed in Cells of HIV–Infected Patients

	CD45RO	CD45RA	CD95	CD25	CD38	HLADR	CD57	CD69	CD7	SLAM
CD4‡	↑	↓	↑	↑	↑	↑	↑	↑	↓	↓
CD8‡	↑	↓	↑	↓	↑	–	↑	↑	–	–

(CD95), CD25, CD38, HLA–DR, CD57, and CD69 and decrease in the subpopulations expressing CD45RA, CD7, and signalling lymphocytic activation molecule (SLAM) ± CD8. T cells increase in the subpopulations expressing CD38, CD45RO, CD57, and CD69 and decrease in the subpopulations expressing CD45RA and CD28 ± NK cells increase in the subpopulations expressing CD56 and decrease in the CD16/CD56 subpopulations HIV infection ultimately results in the appearance of the acquired immunodeficiency syndrome (AIDS); the diagnosis of AIDS was, until the introduction of highly active antiretroviral therapy (HAART), shortly thereafter followed by death [HAASE *et al.*, 1996].

The aim of this study was assessment of HIV infection in individuals infected cells.

Material and methods

This descriptive study was conducted in 2016 in Tehran University of Medical Sciences were on HIV–infected cells.

Control of HIV RNA synthesis is complex and requires the presence of several cellular proteins as well as of viral

transactivators and cis–acting viral elements. Indeed, retroviral long terminal repeats (LTRs) are divided into domains (designated U3, R, and U5) that have distinct functions in transcription either in regulating basal levels or inducing high levels of HIV gene expression [SCHUITEMAKER *et al.*, 1992].

The U3 domain of HIV contains basal promoter elements, including the TATA box for initiation by RNA polymerase II and the site for binding the cellular transcription factor SP1 [COMMITTEE *et al.*, 1994].

Immediately upstream of the core promoter, the virus contains one or more copies of a 10–bp sequence recognized by the enhancer factor nuclear factor (NF)–kB. However, whereas in simple retroviruses regulation of viral transcription is passive (i.e., regulated by cellular factors), in HIV infection this process is more complex and products of the HIV genome are required to achieve high levels of expression [BAGASRA *et al.*, 1996].

Initiation of HIV RNA occurs at the U3/R level (cap site) of the 50 LTR, and the viral transactivator.

The functions through a cis–acting sequence (designated Tat–responsive element, TAR) an RNA encoded by a region located in R, R–U5 is the leader sequence of the full–length and spliced viral transcript, whereas the 30 ends of mRNAs are denied by the R/U5 border in the 30 LTR [PATEL *et al.*, 2008].

Finally, the accessory genes of HIV (vif, vpr, vpu, and nef) (Table 1) are generally denied as dispensable for viral replication based on studies in tissue culture systems.

On the other hand, accessory genes are expressed in vivo and increasing data indicate that they play important roles in the virus–host interplay.

In vivo

The relevant data on mechanisms of HIV replication have been coupled with the results from in vivo studies, thus obtaining a precise understanding of the virus–host relationships [GRANICH *et al.*, 2009].

Indeed, natural history and pathogenicity studies have supplied a prole of HIV activity during the different phases of this infection, have contributed to a better understanding of virus–host interactions, have allowed the application of mathematical models to evaluate the intrahost HIV dynamics, and have provided a theoretical basis for therapeutic antiviral intervention.

Comparative analysis of the sequences of the HIV env gene from a great number of viral isolates has revealed a pattern of has hypervariable regions (designated V1 to V5) interspersed with more conserved sequences in the gp120. This sequence variation consists of mutations (resulting in amino acid substitutions), insertions, and deletions [LAWN *et al.*, 2005].

Among HIV isolates from geographically different locations, gp120 amino acid sequences may diverge up to 20 ± 25 %, whereas other regions of the genome are relatively conserved [LEDERGERBER *et al.*, 2004].

More recently, molecular epidemiology surveys based on env sequences of numerous HIV isolates have revealed at least nine distinct HIV subtypes (or clades) in the acquired immunodeficiency syndrome (AIDS) pandemic, (intersubject HIV variability).

Normal human somatic cells have an intrinsic natural barrier to unlimited cell division [MIRO *et al.*, 2004].

Following a fairly predictable number of cell divisions in culture, most, if not all, mitotically competent human cells reach an irreversible state of growth arrest known as replicative senescence, a process identified by Haybrick in human fetal fibroblasts.

Replicative senescence is a strict characteristic of human cells, and has, in fact, been proposed to constitute a tumor suppressive mechanism [ALTFELD *et al.*, 2001].

Interestingly, experimental cell fusion studies have demonstrated that the property of senescence is genetically dominant over immortality in a variety of human cell types, and spontaneous transformation of human cells in vitro rarely, if ever, occurs [FAWZI *et al.*, 1998].

By contrast, most rodent cells have a high propensity to bypass senescence and transform spontaneously in culture.

The divergent behaviour of human and mouse cells with respect to spontaneous immortalization in vitro suggests that conclusions regarding replicative properties, telomeres, and telomerase drawn from murine studies may not be applicable to human cells.

Identification of the specific telomere sequence led to the rapid development of a variety of techniques to evaluate telomere length.

Telomeres can be measured by isolating total genomic DNA from a population of cells, incubating this DNA with restriction enzymes that digest all but the telomeric and subtelomeric sequences, are performed.

Southern analysis (either gel or slot blot) with radiolabelled telomeric probe [FELLAY *et al.*, 2002] were made.

The results of such analyses provide the mean terminal restriction fragment (TRF) length of a cell population, which, in turn, reacts the composite of the telomere lengths of all the chromosomes in each of the cells in the starting population.

Cell sorting techniques to enrich for particular cell subpopulations [HOGG *et al.*, 1997], analysis of TRF lengths from sorted chromosomes, or probing specific chromosomes containing telomeres that have been seeded with plasmids may, in some cases, increase the precision of the telomere assay.

A second strategy, developed by Landsorp and colleagues, uses fluorescence in situ hybridization (FISH) technology on metaphase spreads or on interphase nuclei, with cytometric visualization and quantitation of the amount of telomere sequences on individual chromosomes [SIMMONDS *et al.*, 1990].

Flow cytometric analysis of telomere size in concert with cell cycle analysis adds an additional dimension to telomere studies [PANTALEO *et al.*, 2004].

Finally, techniques currently under developments that combine FISH telomere analysis with cytometric cell surface phenotype or tetramer–binding will provide critical information on specific subpopulations of cells.

Telomere length assays have contributed remarkable insights into a variety of facets of cell biology in both normal and transformed cells.

The specific telomere sequence also forms the basis for assays to detect telomerase, using techniques involving telomere repeat amplification protocol (TRAP) [SHBATA *et al.*, 1999].

Primary HIV infection can be asymptomatic or can be associated with like syndrome characterized by fever, malaise, and weakness. The early phase of HIV infection is also always correlated with the presence of extremely elevated titers of HIV in the plasma.

A potent HIV–specific immune response is rapidly induced within a few days after primary infection. In this phase, both humoral immunity (HI) and cell mediated immunity (CMI) are promptly stimulated.

It was nevertheless convincingly demonstrated that a significant reduction in HIV plasma viremia depends on the generation of an HIV–specific cell–mediated immune response, independently of the magnitude of the humoral immune response [IMAMI *et al.*, 1999].

This concept is mostly based on the observation that the detection of HIV–specific cytotoxic T lymphocyte (CTL) precedes reductions in viral load, whereas the generation of neutralizing antibodies is delayed and is observed after the changes in HIV viremia has taken place [MAVILIO *et al.*, 2005].

The idea that the modulation of HIV replication is associated with an intact and powerful HIV–specific CMI [KARIM *et al.*, 2010], at least in part derives from the observation that HIV replication is relatively controlled in the initial phases of the infection, when the immune system is powerful and still relatively undamaged.

A long period of clinical asymptomaticity follows primary HIV infection.

This phase is initially characterized by low HIV viral load, reduced HIV replication in peripheral blood mononuclear cells (PBMC), and low HIV isolability from PBMC [LEE *et al.*, 1994].

In this period, HIV replicates vigorously within the lymphatic tissues. CD4 T–helper cell functions are nevertheless often disrupted even in the earlier phases of asymptomatic HIV infection.

The defects detected in T–helper function of asymptomatic, HIV–infected individuals are epitomized by the impairment of the ability of these cells to proliferate and secrete IL–2 in response to nominal antigens [MARKOWITZ *et al.*, 2003].

The T–helper defects observed in this phase of the disease are independent of CD4 counts, and predictive for the subsequent rate of decline in the number of CD4 T lymphocytes, time to onset of AIDS, and time to death.

Abnormalities in T–helper integrity include, besides the defects in IL–2 production, a series of other alterations, among which reduced IL–2 receptor expression, preferential loss of the naive T–cell subset [CROWE *et al.*, 1991], and down–regulation of the expression of the z chain of the T–cell receptor may have an important role in disease progression.

In the period of clinical asymptomaticity, the ability of T lymphocytes to produce cytokines other than IL–2 upon antigenic stimulation is impaired as well, and this impairment appears to be pivotal in the progression of the infection.

To summarize, in vitro stimulation of blood leukocytes from HIV–infected patients results in decreased production of IL–2, IFNg, and IL–12 and in increased production of IL–4, IL–5, IL–6, and IL–10 [LICHTERELD *et al.*, 2004].

Decreased IL–2 production and increased IL–10 generation (and the IL–10/IL–2 ratio) are associated with the isolation of syncytium–inducing (X4) strains of HIV, whereas the opposite scenario (potent type–1 cytokine secretion and weak production of type–2 cytokines) is correlated with a condition of long–term nonprogression [KAPLAN *et al.*, 2001].

Thus, even in the period in which clinical latency is observed, HIV infection is immunologically and virologically extremely active and destructive.

From a virological point of view, the remarkably fast rate of HIV replication, and the enormous ability of this virus to modify in response to exogenous (pharmacologic) and endogenous (immune system) selective pressure, is underlined by the observation that a wild–type, drug–susceptible HIV strain can be totally replaced by a mutant, drug–resistant strain within 2 ± 4 weeks after initiation of antiretroviral therapy [DONAGHY *et al.*, 2003].

The end of the period of clinical latency coincides with the onset of signs and symptoms of AIDS, the rapid destruction of CD4. T lymphocytes and a signi can't increase in HIV viral load.

Results and discussion

The HIV genome encodes for precursor polypeptides of structural and functional virion proteins, regulatory proteins, and other proteins that are dispensable for replication and are called accessory proteins (Table 2).

As for other retroviruses, the genomic HIV RNA is synthesized and processed by the cellular mRNA handling machinery starting from the proviral HIV DNA. For this reason, the viral genome contains a cap structure at the 50 end and a poly–A tail at the 30 end.

Moreover, the diploid lentiviral genome has the additional feature of being rich in A residues (on average 38 ± 39 %). As a direct consequence, the HIV codon usage dramatically from that of cellular genes [DONAGHY *et al.*, 2003].

Control of HIV RNA synthesis is complex and requires the presence of several cellular proteins as well as of viral transactivators and cis–acting viral elements. Indeed, retroviral long terminal repeats (LTRs) are divided into domains (designated U3, R, and U5) that have distinct functions in transcription either in regulating basal levels or inducing high levels of HIV gene expression.

The U3 domain of HIV contains basal promoter elements, including the TATA box for initiation by RNA polymerase II and the site for binding the cellular transcription factor SP1.

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Immediately upstream of the core promoter, the virus contains one or more copies of a 10–bp sequence recognized by the enhancer factor nuclear factor (NF)–kB.

However, whereas in simple retroviruses regulation of viral transcription is passive (i.e., regulated by cellular factors), in HIV infection, this process is more complex and products of the HIV genome are required to achieve high levels of expression.

Initiation of HIV RNA occurs at the U3/R level (cap site) of the 50 LTR, and the viral transactivator Tat functions through a cis–acting sequence (designated Tat–responsive element, TAR), an RNA encoded by a region located in R.

R–U5 is the leader sequence of the full–length and spliced viral transcript, whereas the 30 ends of mRNAs are denied by the R/U5 border in the 30 LTR.

Finally, the accessory genes of HIV (vif, vpr, vpu, and nef) (Table 2) are generally denied as dispensable for viral replication based on studies in tissue culture systems.

On the other hand, accessory genes are expressed in vivo and increasing data indicate that they play important roles in the virus–host interplay [DONAGHY *et al.*, 2003].

Table 2.

Genes of HIV, Proteins, and Function

Essential for Replication	Function	Protein	HIV Gene
Yes	Polyprotein precursor for matrix protein (p17), capsid protein (p24), nucleocapsid protein p9, and p7	Pr55gag	***gag***
Yes	Polyprotein precursor for virion enzymes reverse transcriptase (RT)/RNAse–H (p51), protease (PR) (p10), and integrase (IN) (p32)	Pr160gag–pol	***pol***
Yes	Polyprotein precursor for envelope glycoproteins gp120 and gp41 (receptor binding and membrane fusion, respectively)	gp160	***env***
Yes	Transcriptional transactivator (initiation and elongation of viral transcripts)	p14	***tat***
Yes	Regulates viral gene expression at posttranscriptional levels (regulates splicing and transport of viral RNAs from the nucleus to the cytoplasm)	p19	***rev***
No	Downregulates CD4 receptor, enhances virion infectivity, in¯uences T–cell activation	p27	***nef***
No	Viral infectivity factor (infectivity reduced in vif–minus mutants)	p23	***vif***
No	Virion protein (associated with the nucleocapsid) implicated in regulation of viral and cellular gene expression	p15	***vpr***
No	Influences virus release	p16	***vpu***

In vivo, systemic HIV activity is a formal entity that consists of a sum of dynamic processes, including productive infection of target cells, release of virions outside the infected cell and eventually in the blood compartment, and de novo infection of permissive cells.

The virus variables influencing the level of systemic HIV activity and cell–free virus dynamics include degree of viral expression and host cell range, whereas the host variables include the specific (humoral and cytotoxic) immune response and polymorphism of genes coding for cell receptors of HIV.

The vast majority of quantitative studies carried out in vivo have highlighted the role of cell–free viremia as a reliable index of mean viral activity in HIV infection.

Indeed, viremia–based studies have provided clear evidence that changes in HIV load during the different phases of this infection can be efficiently evaluated

by measuring cell–free virus in plasma samples [YERLY *et al.*, 1999], and that substantial increases in viral load parallel or even predict the disease progression.

These findings have greatly contributed in the last few years to a clearer understanding of the virologic correlates of disease progression, to driving new attempts at understanding the pathogenic potential of HIV, and to designing effective antiretroviral strategies.

Although recent research has highlighted the diagnostic role of other quantitative parameters, including viral transcription pattern and provirus copy numbers, and although in some cases virus compartmentalization may influence the exact correspondence between cell–free plasma viremia and systemic viral activity, the analysis of viral genome molecules in plasma samples is still a major molecular correlate of systemic viral activity at the level of the whole body in many human viral infections.

The evaluation of patients undergoing potent antiviral treatments has allowed the dynamics of cell–free virus in plasma to be addressed in vivo [CARMICHAEL *et al.*, 1993].

Importantly, these studies have documented the dynamics of cell–free virions in plasma (half–life being approximately 5.7 h) and the turnover of infected cells.

Furthermore, the sensitivity and specificity performances of most quantitative molecular methods have provided in the last few years a simple approach to the evaluation of gene transcription in vivo and in vitro.

In HIV infection, consistent evidence has indicated that progression of disease is driven by an increase in viral load evaluated as cell–free plasma virus.

To address whether this increase is contributed by the dysregulation of the molecular mechanisms governing virus gene expression at the transcriptional or post–transcriptional levels, several quantitative virologic parameters (including provirus transcriptional activity and splicing pattern) have been analysed in subjects with nonprogressive HIV infection and compared with those of matching groups of progress or patients.

It was observed that high levels of unspliced (US) and multiply spliced (MS) viral transcripts in peripheral blood mononuclear cells (PBMCs) correlate with the decrease in CD4. T cells [PAIARDINI *et al.*, 2005] following the general trend of systemic HIV–1 activity, but also that MS mRNA levels in PBMCs are closely associated with the number of productively infected cells, because the half–life of this class of transcripts after administration of a potent protease inhibitor is very consistent with that of productively infected cells.

The transcriptional pattern observed during in vitro subsequent analysis has revealed that both linear and conformational determinants influence the functional and antigenic structure of the gp120; this is a crucial pathogenic issue, in as much as all neutralizing antibodies are directed against env–encoded domains in HIV–infected hosts. Indeed, infections with retroviruses are also characterized by different (from moderate to high) levels of intrahost viral genetic variation.

This viral variability is dependent upon mutation, recombination, degree of viral replication, and the host's selective pressure [KAUFMANN *et al.*, 2005].

In HIV infection, the viral population is represented by related, nonidentical genetic variants, designated quasispecies. The error–prone nature of the HIV reverse transcriptase (RT) and the absence of a 30– exonuclease proofreading activity determine in vitro about 3–10 mutations per nucleotide per replication cycle.

Although the mutation rate observed in vivo is lower than that predicted from the deity of pureed RT (because a number of newly generated variants are unable to replicate or are cleared by the host's immune system) the viral replication dynamics and the host's selective forces determine a continuous process of intrahost HIV evolution [STAPRANS *et al.*, 1995, CHIURCIU, *et al.*, 2017, MUSELIN, *et al.*, 2014].

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Overall, the data currently suggest that viral genetic variability is the molecular counterpart of a continuous dynamic interplay between viral (i.e., HIV–1 replication dynamics and generation of variants by mutation and recombination) and host factors (i.e., selective pressure).

In this context, intrahost evolution of HIV–1 populations may be compatible with a Darwinian model system, as recently suggested [AOKI–SEI *et al.*, 1992].

The complete elucidation of the mechanisms driving intrahost HIV–1 evolution is of crucial importance for understanding the natural history of this infection and developing effective anti–HIV strategies.

In particular, the envelope glycoproteins of HIV–1 interact with receptors of the target cells and mediate the process of virus entry.

This process is complex, including binding of the viral gp120 to CD4, conformational changes of the viral glycoprotein, and subsequent use of a co-receptor before gp41–mediated fusion of the viral envelope and the cellular membrane (Table 3).

The evolutionary changes characterizing the HIV–1 population during the natural history of infection strongly influence crucial regions of the viral env gene.

Because different variable domains of the HIV–1 gp120 play a key role in driving the early steps of the viral infection cycle, including co-receptor usage and CD4 independence, careful analysis of the intrahost evolution of the HIV–1 env gene is strategic for addressing the relevant features of the virus–host relationships [GOTTLIEB *et al.*, 2002].

In addition, HIV entry is at present an attractive target for new classes of antiretroviral compounds; at present, these compounds include inhibitors of HIV binding to CCR5 and CXCR4 co-receptors and fusion inhibitors.

The V3 sequence is a variable domain in the HIV gp120 and contains 35 amino acids arranged in a loop. This domain plays a crucial role in driving important biological properties of the virus, including cell tropism.

Generally, mutations in the V3 loop do not affect the ability of gp120 to interact with the CD4 receptor, although several studies have unambiguously indicated that V sequences play an important role in two correlated biological features with pathogenic implications, that is, syncytium formation and co-receptor usage [PILCHER *et al.*, 2004].

Importantly, analysis of chimeric viruses has revealed that changes in the V3 loop can convert a nonsyncytium inducing (NSI), slowly replicating virus into a syncytium inducing (SI), and rapidly replicating virus.

Table 3.

HIV Cell Receptors and Their Natural Ligands

BONZO	BOB	CCR2b	CCR3	CXCR4	CCR5	CD4	HIV
+	+	+	+		+	+	**NSI**
+	(+)	+	+	+	+	+	**SI**
?	?	MCP–1, MCP–2, MCP–3	RANTES, MCP–3, EOTAXIN	SDF–1	MIP–1a, MIP–1b, RANTES, MCP–4	MHCCl. II	**Natural ligands**

**NSI, nonsyncytium inducing; SI, syncytium inducing.*

In the context of HIV disease, telomere length analysis has been used as a tool to analyse the dynamics of lymphocyte homeostasis and its relation to ultimate immune collapse that is fundamental to HIV disease pathogenesis.

Research in this area has provided extraordinary insights into both T and B function in HIV–infected persons, as will be described below.

In a longitudinal analysis of peripheral blood mononuclear cell (PBMC) samples collected over a period of 9 years, Miedema and colleagues

documented those telomere lengths of total PBMC from HIV–infected individuals shortened at an accelerated rate compared with age–matched seronegative controls.

For HIV–infected individuals, the mean TRF length loss in the progressors (175G105 bp/yr) was greater than that in the asymptomatic individuals (114G100 bp/yr), and both were significantly increased compared with healthy controls (4.7G71 bp/yr).

These data indicate that increased cell division of immune cells is associated with HIV infection and correlates with disease status.

Telomere length measurements on denied cell populations separated by cell sorting procedures have provided further insight into HIV disease pathogenesis.

Several groups compared telomere lengths of the two T–cell subsets.

In four independent studies, telomere shortening was consistently observed within the CD8 T–cell subset, with only minimal or no telomere shortening in the CD4 subset.

In one study, sequential samples from several individuals demonstrated progressive CD8 T–cell telomere shortening over time.

In another study, the CD8 T cells were further sorted; permitting the demonstration that within the CD8 subset, all of the telomere shortening could be accounted for by the cells that lacked CD28 expression.

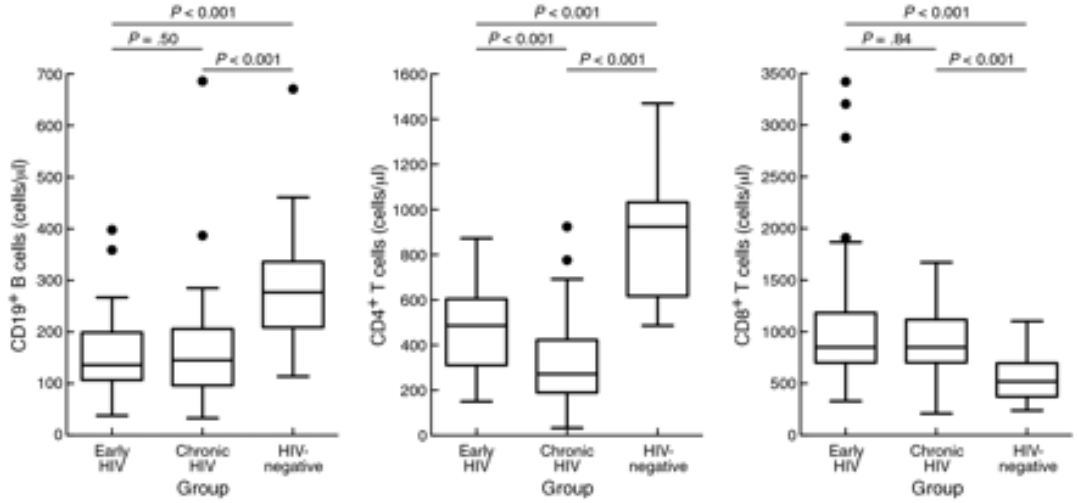

Figure 1. Lymphocyte counts in healthy individuals and early and chronically HIV–infected individuals before ART. Box plots of absolute lymphocyte numbers in the peripheral blood are shown for 3 groups of individuals: early and chronic HIV–infected individuals before initiation of therapy as well as HIV–uninfected individuals. Box plots include median with 25th and 75th percentile boarders and error bars represent 10th and 90th percentiles.

B–cell and CD4+ T–cell counts were significantly higher in HIV–negative individuals compared with both early and chronically HIV–infected individuals whereas CD8+ T–cell counts were lower in HIV–uninfected compared with both HIV–infected groups (Figure 1).

In fact, the telomere lengths of the CD8 and CD28 T cells were the same size as those of centenarian lymphocytes, suggestive of accelerated immunological aging during HIV disease.

Boussin and colleagues have also documented CD8 telomere shortening and, furthermore, provide data showing that B–cell telomeres shorten as well, consistent with the well–documented hyperactivation and polyclonal antibody production observed during HIV infection.

Finally, a novel approach to telomere analysis in the two T–cell subsets was used by Hodes and colleagues in an effort to eliminate the variability inherent in telomere lengths in

outbred human populations by comparing cell populations derived from HIV–discordant identical twins.

These studies showed that the mean TRF of the CD8 T cells was shorter in HIV infected versus uninfected twins (mean difference 1.1 ± 0.7 kb).

Thus, accelerated telomere shortening in CD8 T cells during HIV disease has been an unequivocal observation in all of the published studies that have used this experimental approach.

Numerous studies have been performed in an effort to elucidate CD4 T–cell dynamics in HIV disease, but, in marked contrast to the CD8 T–cell findings, the results have been inconsistent, showing an entire spectrum of changes, possibly suggesting a more complicated picture.

For example, in the same twin study in which CD8 T–cell telomeres were shorter in the infected twin [PILCHER *et al.*, 2004, DUMITRESCU, *et al.*, 2014, CERNEA, *et al.*, 2015].

Demonstrated the CD4 T cells in the HIV–infected twins were longer than those in the uninfected twin (mean difference 0.9 ± 0.4 kb).

Furthermore, the CD4 T–cell subset showed no difference in the total number of population doublings achieved in long–term culture between the HIV–infected versus uninfected twins.

The data from these studies were interpreted to indicate that the immune deficiency associated with HIV disease could not be attributed to exhaustion of the replicative potential of CD4 T cells.

Even in studies that do document some CD4 T–cell telomere shortening, these changes do not correlate with markers of disease progression.

Preliminary evidence from two studies had suggested that CD4 telomere shortening might occur at late stage disease. However, in a subsequent more extensive analysis of TRF in PBMC versus CD4 T cells, both increases and decreases of CD4 T–cell telomeres were observed, with no correlation between CD4 T–cell telomere length changes and other well–established markers of disease status, such as CD4 counts and CD8.CD38. T cells [PILCHER *et al.*, 2004].

Immune activation was early recognized as being a characteristic feature of HIV infection.

Because HIV replicates in activated cells [APPAY *et al.*, 2000], the observation that an abnormal and augmented immune activation takes place in this disease has an obvious, immediate clinical relevance.

Additionally, immune–activated cells will be unable to respond to antigens, might produce reduced amounts of soluble antiviral factors, and are more susceptible to programmed cell death.

A vast array of immune cells can phenotypically be characterized as being activated in HIV–infected individuals and activation is seen in virtually each immune cell compartment.

Thus, CD4 and CD8 T lymphocytes as well as natural killer (NK) cells show signs of immune activation. Markers of T–cell activation in HIV infection include, among others, increased cell surface expression of CD45RO, CD25, CD28, CD38, HLA–DR, and CD95 [APPAY *et al.*, 2000].

Table 4.

Alterations of the Fas/Fas Ligand (FasL) and Soluble APO–1/Fas (sAPO–1/Fas) System in HIV Infection

*Up–regulation of Fas on lymphocytes of HIV–infected individuals
*Augmented susceptibility of lymphocyte of HIV–infected individuals to apoptosis upon ligation of Fas
*Correlation between expression of Fas/susceptibility of Fas ligation to apoptosis and clinical stage of HIV infection
*Monocyte–CD4/Fas–FasL ``kiss of death''
*CD4–CD8/Fas–FasL ``kiss of death''
*Reduced serum concentration of sAPO–1/Fas in progressing HIV infection

Fas and FasL thus reducing apoptosis [HARARI *et al.*, 2004]. CD95 and its ligand are expressed at high levels on activated mature lymphocytes, suggesting that CD95 expression and subsequent cell death may be the physiological consequence of cellular activation.

Surface expression of CD95 is increased on lymphocytes of HIV–infected individual.

Additionally, the interaction of CD95 with its ligand in HIV–infected cells provokes apoptosis in a high percentage of lymphocytes.

Up–regulation of CD95 on monocytes is observed as well in HIV infection; the augmented expression of CD95 on these cells could be responsible for the destruction of uninfected CD4 T lymphocytes subsequent to antigenic presentation and interaction between CD95 (on monocytes) and CD95L (on CD4 cells), via a ''kiss of death" mechanism [KELLEY *et al.*, 2009].

In addition to mechanisms of accelerated suicide or paracrine death, CD95–induced apoptosis during HIV infection may be mediated by cytotoxic elector cells.

The magnitude of the CTL response during HIV infection and the high proportion of CD95 compliant target cells among patients' lymphocytes argue for a deleterious role of CTL against activated T cells.

In the contest of HIV pathogenesis, the persistence of continuously activated antiviral CTL would thus be associated with CD95L expression and, therefore, with the ability to kill both virus–infected cells and noninfected, activated compliant CD95 Target cells.

The exacerbation of the CD95 system would drive antiviral CTL in a deleterious role and particularly in the physiological elimination of CD4 T cells, irrespective of the infected status.

This would contribute to the collapse of the immune system.

Conclusions

Expanded analysis of the molecular biology of HIV has been the key to understanding the mechanisms by which this virus persists in the host and causes AIDS, and to developing active antiretroviral strategies.

Application of powerful molecular biology tools has allowed researchers to obtain fundamental results on many aspects of HIV biology in vitro (i.e., in cell–free and tissue culture systems) and in vivo (i.e., directly in samples from the susceptible host).

Importantly, knowledge of the molecular mechanisms in each step of the virus life cycle has provided an essential basis for discovering new antiviral compounds.

Otherwise, understanding of the relevant features of both the HIV turnover in vivo and the intrahost HIV evolution is crucial for developing active anti–HIV strategies.

Indeed, the HIV biology poses several challenges to the development of these strategies. In particular, sequence variation resulting from errors of the viral RT and recombination renders HIV an elusive target for both antiviral compounds and vaccines.

In this context, novel diagnostic molecular tools to control development of viral resistance to the different classes of antivirals and new effective therapeutic approaches, including genetic and immunologic strategies, could be the key to inhibiting HIV replication in the future.

Telomere studies have highlighted a previously unrecognized aspect of CD8 T–cell biology occurring during HIV disease.

The demonstration that the nonproliferative CD28, CD8 T–cell supbpopulation with telomeres in the identical 5 ± 7 kb range, previously associated with replicative senescence reaches levels of > 65 % in HIV–infected persons which further underscores the biological relevance of these observations.

In light of the increasing recognition of the importance of the protective role of CD8 T–cell in HIV disease, it seems clear that research aimed at retarding CD8 T–cell telomere shortening and delaying

replicative senescence might lead to novel immunotherapeutic strategies that will complement treatments aimed at the virus itself.

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Conflict of interest statement

We declare that we have no conflict of interest.

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