

# Mechanisms of Virologic Control and Clinical Characteristics of HIV+ Elite/Viremic Controllers

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Human immunodeficiency virus type 1 (HIV-1)† disease is pandemic, with approximately 36 million infected individuals world-wide. For the vast majority of these individuals, untreated HIV eventually causes CD4+ T cell depletion and profound immunodeficiency, resulting in morbidity and mortality. But for a remarkable few (0.2 to 0.5 percent), termed elite controllers (ECs), viral loads (VLs) remain suppressed to undetectable levels (< 50 copies/ml) and peripheral CD4+ T cell counts remain high (200 to 1000/μl), all in the absence of antiretroviral therapy (ART). Viremic controllers (VCs) are a similar but larger subset of HIV-1 infected individuals who have the ability to suppress their VLs to low levels. These patients have been intensively studied over the last 10 years in order to determine how they are able to naturally control HIV in the absence of medications, and a variety of mechanisms have been proposed. Defective HIV does not explain the clinical status of most ECs/VCs; rather these individuals appear to somehow control HIV infection, through immune or other unknown mechanisms. Over time, many ECs and VCs eventually lose the ability to control HIV, leading to CD4+ T cell depletion and immunologic dysfunction in the absence of ART. Elucidating novel mechanisms of HIV control in this group of patients will be an important step in understanding HIV infection. This will extend our knowledge of HIV-host interaction and may pave the way for the development of new therapeutic approaches and advance the cure agenda.

## INTRODUCTION

### *Natural Progression of HIV Infection*

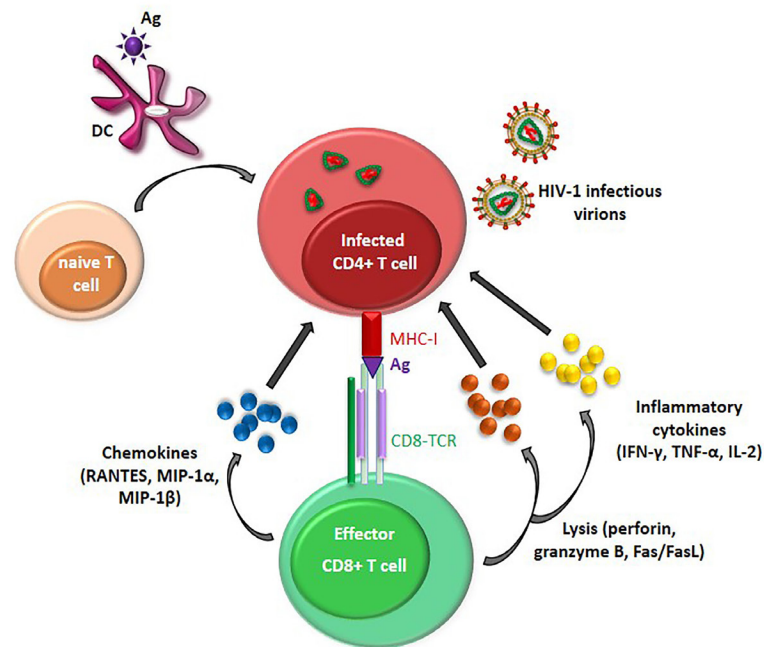
Human immunodeficiency virus-1 (HIV-1) is transmitted via hetero or homosexual contact, exchange of infected blood via transfusion and/or the sharing of nec-

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†Abbreviations: HIV-1, Human immunodeficiency virus-1; DC, dendritic cell; CD4, cluster of differentiation 4; CCR5, C-C Motif Chemokine Receptor 5; TH, T helper; MIP-1, macrophage inflammatory protein-1; RANTES, Chemokine (C-C motif) ligand 5; CXCR4, C-X-C chemokine receptor type 4; CV, cardiovascular; ART, antiretroviral therapy; env, envelope; dscDNA, double-stranded cDNA; PIC, pre-integration complex; LTR, long terminal repeat; TRIM-5α, tripartite motif-containing protein-5 alpha; APOBEC, apolipoprotein B mRNA editing enzyme catalytic; EC, elite controller; VC, viremic controller; LTNP, long-term non-progressor; LTNP 7, LTNP through 7 years of follow-up; LTNP 10, LTNP through 10 years of follow-up; NC, non-controller; VL, viral load; AIDS, acquired immune deficiency syndrome; HLA, human leukocyte antigen; TNF-α, tumor necrosis factor-alpha; IL-2, interleukin-2; IFN-γ, interferon-gamma; CDK, cyclin dependent kinase; CTLA-4, Cytotoxic T-Lymphocyte Antigen-4; NK, natural killer; ZNRD1, zinc ribbon domain; RNF39, ring finger protein 39; GWAS, Genome-wide association studies; SNP, single nucleotide polymorphisms; LPS, lipopolysaccharide; Mx2/McB, myxovirus resistance protein 2; NFAT, nuclear factor of activated T-cells; BST-2, bone marrow stromal antigen 2.

Keywords: HIV, Elite Controllers, Viremic controllers, Long-term non progressors

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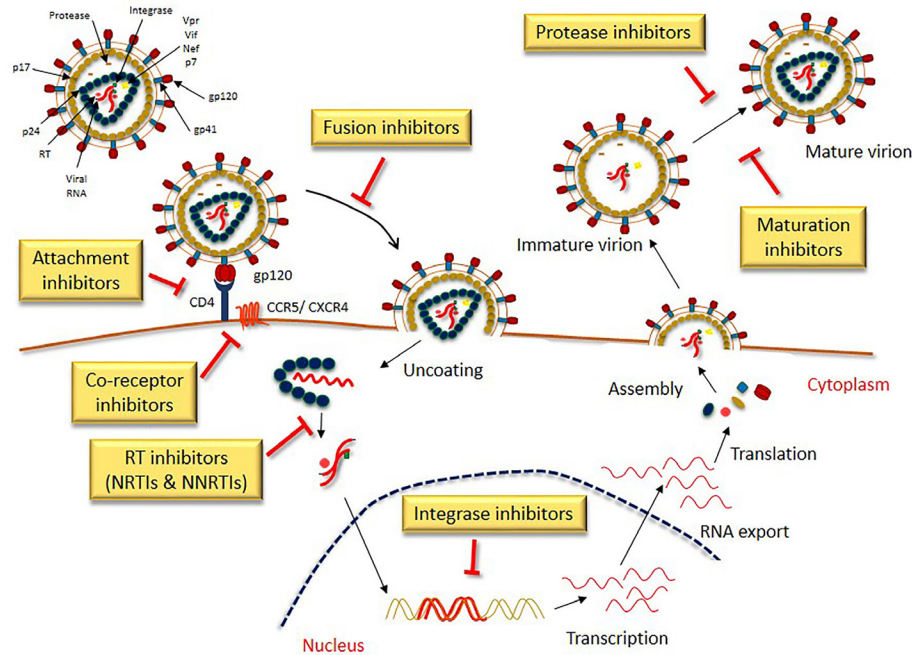


**Figure 1.** T cell immune responses after HIV-1 infection.

dles, breast-feeding from an infected mother to child, or trans-placentally from an infected mother to fetus [1,2]. After introduction by sexual contact, virus encounters Langerhans cells, antigen-presenting dendritic cells (DCs) that densely populate mucosal surfaces of the gut and vaginal tissue. And although these cells express low levels of cluster of differentiation 4 (CD4) and C-C Motif Chemokine Receptor 5 (CCR5) on the cell surface, they actively bind to HIV particles via these and other receptors, and facilitate attachment and fusion of viral and host cell membranes [3,4]. Langerhans cells with bound, internalized virus migrate from the mucosal surface to infect neighboring T cells expressing CD4 and CCR5 receptors, before arriving at regional lymph nodes [5]. In addition, these cells are important for priming naive CD4<sup>+</sup> T cells into HIV-specific T helper ( $T_H$ ) cells. Local viral replication then occurs, followed by initial detectable plasma viremia, a process called primary infection [4]. Once within regional and draining lymph nodes, infected CD4<sup>+</sup> T cells induce T cell activation and proliferation by stimulating HIV-specific CD8<sup>+</sup> T cells [6] (Figure 1). The key effector functions of HIV-specific CD8<sup>+</sup> T cells are three-fold and include (i) T cell receptor-based recognition of virally-infected cells and subsequent release of perforins and granzyme that are essential to cytotoxic function, (ii) prevention of viral entry via release of competitively binding chemokines such as macrophage inflammatory protein-1 (MIP-1) $\alpha$ , MIP-1 $\beta$  and Chemokine (C-C motif) ligand 5 (RANTES), that serve as chemo-attractants for lymphocytes and monocytes [7,8] and (iii) inhibition of viral rep-

lication via production of cytokines and activation of the interferon (IFN) signaling pathway [9] (Figure 1).

During HIV infection, chemokine ligands inhibit viral entry into cells, preventing viral replication and delaying disease progression by competitively binding to the co-receptors, CCR5 and C-X-C chemokine receptor type 4 (CXCR4), on CD4<sup>+</sup> T cells and macrophages [10,11]. The polyfunctional HIV-specific and non-specific CD8<sup>+</sup> T cell responses induce cytotoxic killing of HIV-infected cells [12] and establish a general antiviral state by enhancing innate immunity, making cells more resistant to virus replication. This, as well as gastrointestinal microbial translocation which occurs due to depletion of gut-associated lymphoid tissue secondary to early, massive HIV replication, creates a pro-inflammatory environment that drives chronic immune activation and leads to disease progression [13]. One consequence of this, with respect to long-term clinical outcomes in HIV-infected individuals, may be a greater propensity for the development of cardiovascular (CV) and other inflammatory diseases [14]. Even in the presence of antiretroviral therapy (ART) there are low levels of viremia; whether there remains active viral replication in blood and lymphoid tissue is controversial and subject to debate and continued study [15]. But presumably the presence of virus or viral gene products is at least in part responsible for the chronic inflammatory state, which may result in a myriad of untoward consequences, with adverse effects on the health of the infected individual.



**Figure 2.** HIV-1 viral cycle and FDA-approved therapies and targets (attachment and maturation inhibitors in late stage clinical trials).

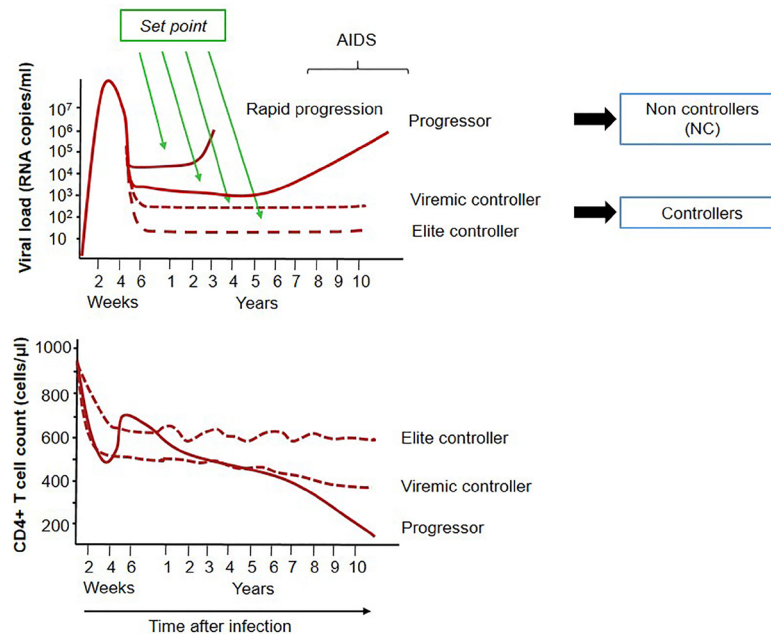
### HIV-1 Viral Life Cycle and Antiretroviral Therapy

The viral life cycle begins when the envelope (env) glycoprotein gp120 binds to the cell surface receptor CD4 and the membrane co-receptors CCR5 or CXCR4 [16,17] (Figure 2). After fusion of the viral and cellular membranes, the viral particle enters into the cytosol and viral RNA is reverse transcribed into proviral double-stranded cDNA (dscDNA) [18]. Although it is not clear whether reverse transcription occurs within an intact viral capsid core, some studies suggest that post-entry at least a partial capsid core structure is required for optimal reverse transcriptase activity [19,20]. After formation of a pre-integration complex (PIC), dscDNA is imported into the cell nucleus through an intact nuclear pore [21] and the genetic material either circularizes as one or two long terminal repeat (LTR)-containing circles (considered dead-end products) or becomes incorporated irreversibly into the host genome via the catalytic activity of viral integrase [22]. Transcription of integrated provirus yields viral mRNAs of different sizes, which are exported from the nucleus [23,24]. These mRNAs serve as templates for protein production and genome-length RNA is incorporated into nascent viral particles, likely cooperatively assembled at the plasma membrane [25]. Finally the newly made viral particles bud from the plasma membrane and mature through the activity of the viral protease, which cleaves the Gag and Pol polyprotein, to produce fully infectious particles [26].

*In vitro* approaches have identified a number of host

genes that negatively regulate or interfere with virus replication. These potent HIV restriction factors include tripartite motif-containing protein 5 alpha (TRIM-5 $\alpha$ ) [27], multiple apolipoprotein B mRNA editing enzyme catalytic (APOBEC) family members [28], the nucleotide hydrolase SAMHD1 [29], SERINC family members, myxovirus resistance protein (MXB), and tetherin [30-32]. Each of these factors acts at distinct steps of the virus lifecycle to inhibit viral replication and yet none has been definitively implicated in viral control in humans [27,30]. On the other hand, many steps of the viral life cycle are targets for ART [33] (Figure 2), and one of the greatest success stories of the last two decades of modern medicine is the widespread use of ART to treat HIV and transform the infection, once considered a death sentence, into a chronic, very manageable disease. Despite this, ART is life-long and non-curative, and once therapy is stopped or drug resistance develops, viral rebound invariably occurs within weeks and CD4 counts then decline [34,35].

As discussed above, HIV-1 infects both activated and resting cells, allowing the viral genome to be permanently integrated into the chromosome of a host T cell or tissue macrophage, cell types that can be very long-lived [36]. Latent, cellular reservoirs of virus are established very early during primary infection, even in the presence of ART, and their very long half-life and consequent slow decay constitutes the major barrier to eradication [37]. Thus, despite the extraordinary advances that have been made in ART over the last two decades, we still have



**Figure 3.** Progression of disease after HIV-1 infection in HIV-1 progressors, EC and VC.

much to learn regarding how to effectively control and eventually eradicate the virus. Identifying novel mechanisms for HIV control bears critical importance to HIV research and treatment, as it will extend our knowledge of the HIV-host interaction and potentially pave the way to new therapeutic approaches.

#### *Elite Controllers, Viremic Controllers, and Long-term Nonprogressors*

Elite controllers (ECs) are a small subset of HIV-1 infected individuals (on the order of 1 in 200 to 1 in 500 or 0.2 to 0.5 percent) who have the ability to suppress viremia to undetectable levels ( $< 50$  copies/ml), while maintaining elevated CD4 cell counts (200 to 1000/ $\mu$ l) in the absence of ART [38-42] (Figure 3). These ECs have been intensively investigated over the last several years in order to determine how they are able to naturally control HIV. A similar subset of HIV-infected individuals termed viremic controllers (VCs) achieve a lesser degree of virologic control ( $200 < VL < 2000$  copies/ml), while also maintaining elevated CD4 cell counts (typically  $\leq 500$ / $\mu$ l), in the absence of ART [43]. ECs and VCs are part of a significantly larger cohort of HIV-infected persons, described as long-term non-progressors (LTNP). LTNP are characterized by their ability to maintain elevated CD4 cell counts in the absence of ART [43].

These individuals can be identified early during the course of HIV infection and achieve a significantly lower VL set point after sero-conversion [4,44-46]. As a result, collectively, ECs, VCs and LTNP, hereafter termed

“controllers,” have more favorable outcomes compared to most HIV-infected individuals who do not have ability to achieve virologic suppression in the absence of ART, termed “non-controllers” (NC) [43]. Although clinical latency in untreated non-controllers may persist for years, because of unrelenting high level viral replication for the vast majority of these patients there is an inexorable loss of CD4+ T cells and immune system decline, eventually resulting in acquired immune deficiency syndrome (AIDS) [47] (Figure 3).

Little is known regarding the precise mechanisms that allow robust control of HIV infection, especially in ECs/VCs. Further investigation into how controllers achieve such a high degree of virologic control may help facilitate efforts directed towards a “functional cure” for HIV, in which the virus is still present in latent reservoirs but never reaches high levels of replication, all in the absence of ART.

#### *Epidemiology and Clinical Definitions of Elite Controllers*

As mentioned above the prevalence of the ECs phenotype has been estimated to be  $< 0.5$  percent of the HIV positive patient population [48,49]. The prevalence estimates for VCs have not been entirely elucidated, but are believed to be several-fold higher compared to ECs [43]. These prevalence estimates for ECs reflect data gathered from studied HIV-1+ cohorts within the U.S and Europe. Little is known regarding the prevalence of these controller phenotypes in Sub-Saharan Africa and Asia. In gener-

**Table 1. Viral proteins and host restriction factors implicated in control in HIV-1 VCs/ECs.**

Viral protein/ Host factor	Mechanism of action	[ref]
<i>nef</i>	<ul style="list-style-type: none"> <li>Downregulates surface levels of MHC-I and MHC-II</li> <li>Modulates TCR signaling by inducing/ blocking NFAT and IL-2 production in fresh/ activated T cells, respectively</li> <li>Prevents incorporation of SERINC-3 and SERINC-5 into HIV-1 virions, enhancing infectivity of the virus</li> </ul>	[32,138-140]
<i>vpu</i>	<ul style="list-style-type: none"> <li>Downregulates CD4, and BST-2/tetherin</li> </ul>	[30,141,142]
<i>vif</i>	<ul style="list-style-type: none"> <li>Binds to and blocks the antiviral activity of APOBEC3 proteins, in conjunction with other host factors, inducing their proteasomal degradation</li> </ul>	[143]
<i>TRIM-5<math>\alpha</math></i>	<ul style="list-style-type: none"> <li>Binds to and multimerizes on the viral capsid, somehow inhibiting viral replication</li> <li>Initiates innate immune sensing of cytosolic viral capsid</li> <li>Counteracted by mutations in viral capsid</li> </ul>	[27]
Mx2/MxB	<ul style="list-style-type: none"> <li>Delays HIV-1 DNA nuclear import and integration by targeting viral capsid, exact mechanism of action uncertain</li> <li>Counteracted by mutations in viral capsid</li> </ul>	[31,144]
APOBEC3 family members	<ul style="list-style-type: none"> <li>Inhibits viral reverse transcription and integration</li> <li>Induces lethal mutations in viral cDNA</li> <li>Counteracted by <i>vif</i> (see above)</li> </ul>	[28]
Tetherin	<ul style="list-style-type: none"> <li>Inhibits HIV-1 release by binding virus particles that bud through the cell membrane</li> <li>Counteracted by <i>vpu</i> (see above)</li> </ul>	[30,145]
Serinc-3/5	<ul style="list-style-type: none"> <li>Inhibit HIV-1 particle infectivity</li> <li>Counteracted by <i>nef</i> (see above)</li> </ul>	[32]

MHC: major histocompatibility complex; TCR: T Cell Receptor; NFAT: nuclear factor of activated T-cells; BST-2: bone marrow stromal antigen 2; APOBEC: apolipoprotein B mRNA editing enzyme 3 catalytic polypeptide; Mx2/McB: myxovirus resistance protein 2; BST-2: bone marrow stromal antigen 2.

al, there are no consistent demographic patterns among controllers, with respect to gender or race predilection, or differences in modes of HIV transmission between controllers and NCs [43].

As the sensitivity of VL assays improved over time, the definition of who is classified as an ECs has also changed. For example, an individual characterized as having an undetectable VL between 1995 and 2000 (VL of < 500 copies/ml) may not be identified as an ECs today, as the VL assays in routine clinical use are now able to detect less than 20 copies/ml. This is a potential confounder of longitudinal analyses that evaluate clinical outcomes over prolonged periods of time that cross generations of VL assays.

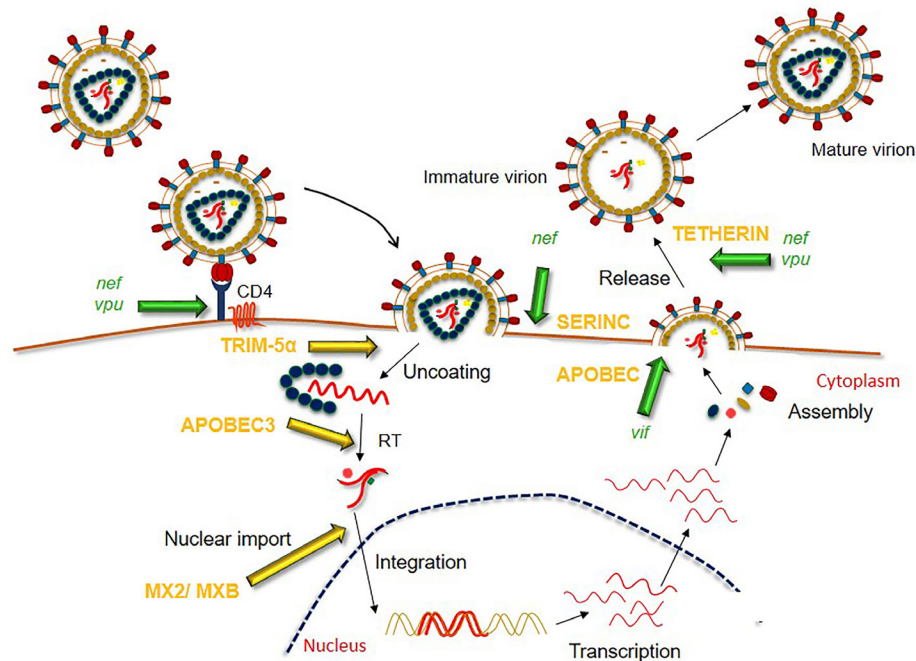
Furthermore, fluctuations in HIV VL are observed naturally during the course of HIV infection and are usually due to concurrent illness or other co-morbidities, receipt of vaccinations, variability or reproducibility of the assay, or inconsistent ART compliance (of course the latter does not pertain to those off therapy) [43]. The ability to not only achieve undetectable VLs, but to sustain them is what differentiates an EC from an NC. Therefore, widely accepted study definitions of an EC include

having three or more VL determinations below the limit of assay detection (usually < 50 copies/ml), spanning 12 months or more, in the absence of ART [43,50,51]. Other studies have required a high percentage of VL values below the limit of detection (> 90 percent) over 10 years to define an EC, although these metrics have not been widely employed [48]. VCs are similar, except that 200 < VL < 2000, with an occasional higher or lower value.

## MECHANISMS OF CONTROL OF HIV-1 IN ELITE CONTROLLERS

### *Viral and Host Cell Intrinsic Factors*

Some studies have suggested that the viral control is the result of infection with defective viral strains [52,53]. However, other studies have proposed that the majority of control is due to host factors. A summary of the viral proteins and host restriction factors implicated in control in VCs/ECs and their role in viral cycle is included in Table 1 and Figure 4. Infection with highly attenuated HIV was observed in a group of recipients of blood products from a common infected donor. The transmitted virus contained a deletion in the viral accessory gene *nef*, and



**Figure 4.** Host restriction factors and lentiviral proteins in HIV replication.

all recipients maintained virologic suppression for years [54]. This result contributed to the belief that attenuated virus played a potentially major role in achieving the controller phenotype. Eventually, however, many of these infected individuals progressed to AIDS, thus minimizing the role of defective HIV in the controller phenotype [55].

Several reports have concluded that mutations within HIV accessory genes can lead to virus control and disease non-progression [56,57]. These studies support the idea of a relative attenuation of viral protein function in EC-derived HIV sequences. This includes a possible role of mutated HIV *vif* gene in reduced viral replication in ECs [58,59]. Additionally, the presence of a premature stop codon and a rare mutation in HIV *nef* and *vif* genes, respectively, was only observed in some EC patients [60]. Also, infection with attenuated forms of HIV-1, including deletions in *nef*, contributed to the absence of disease progression in a subset of patients [61,62], and reduced *nef* function has been shown in some ECs compared to progressors [63]. Recently, a modestly reduced function of HIV gene *vpu* has been observed in a subgroup of ECs [64]. Most certainly, in chronically infected patients, even ECs, there is a wealth of genetic diversity in HIV sequences, both in plasma and archived as integrated provirus, and how much that contributes to virologic control or viral load set point is highly variable and likely very patient-dependent.

Whether and to what extent that viral genetic diversity contributes to the controller phenotype remains a mostly unanswered question, especially since the *in vivo*

data is strictly correlative. In some ECs, certain polymorphisms within the HIV genome were likely acquired early during the course of infection, rendering the virus somewhat devoid of genetic variability and thus yielding a relatively poorly replicating virus [53,65]. Many of these studies involved small numbers of ECs in whom replication competent viruses were not isolated, thus limiting the generalizability of the conclusions. Other work has isolated and analyzed the genomes of replication-competent virus from ECs and results have shown comparable degrees of genetic variation, replication, and evolution, compared to virus isolated from NC [52]. Thus, perhaps host factors play a more significant role in achieving and sustaining virologic control. Consistent with this idea, Buckheit et al., were able to isolate identical viruses from NC and one EC and another VC, consistent with host factors having a dominant role in the control of the HIV-1 replication [66].

### Cellular Immune Responses

Studies have suggested that viral control is strongly correlated with the cellular and humoral immune responses in man [67]. A tight association has been observed between Gag-specific cytotoxic T lymphocyte responses and viral control [68,69], and most notably, HIV-1 specific-CD8<sup>+</sup> T cell responses against viral structural proteins have been shown to correlate inversely to set point levels of viral RNA [69]. More recently, greater avidities of Gag-specific T cell and human leukocyte antigen (HLA)-B-restricted responses were seen *in vivo* in

**Table 2. Genetic alleles associated with HIV control.**

Genes	Author	Journal, year [ref]
HLA-DRB1*13	Malhotra, U. et al Chen, Y. et al	J Clin Invest, 2001 [146] Hum Immunol, 1997 [147]
MICB, TNF, RDBP, BAT1-5, PSORSICI, HLA-C	Limou, S. et al	J Infect Dis., 2009 [148]
HLA-B57, HLA-C	Fellay, J. et al Trachtenberg, E. et al	Science, 2007 [105] Genes Immun, 2009 [107]
HLA-B57, HLA-B27	Pereyra, F. et al	Science, 2010 [51]
HLA-DRB1*13 and/or HLA-DRB1*06	Ferre, AL. et al	J Virol, 2010 [149]
HCP5, HLA-C	Han, Y. et al	AIDS, 2008 [108]
HLA-B57	Tang, Y. et al Migueles, SA. et al Gao, X. et al Kiepiela, P et al Bailey, J.R. et al	AIDS, 2010 [109] J virol, 2003 [104] Nat Med, 2005 [150] Nature, 2004 [102] J Exp Med, 2006 [103]
HLA-A, HLA-B, CCR3	McLaren, P.J. et al	PNAS, 2015 [151]

ECs than in NC [70], consistent with these HIV-specific CD4+ and CD8+ T cell immune responses occurring more frequently in ECs than NCs [71,72].

On the other hand, the absence of some of these HIV-specific CD4+ T cell responses has been shown to be a marker of disease progression [73]. CD8+ T cells from ECs have exhibited more polyfunctional capabilities in response to HIV antigens compared to NC, with greater degranulation and release of perforin and granzyme B [74-76]. Furthermore, CD8+ cells from HLA-B\*57/5801 ECs were more efficient at eliminating potentially infected resting and activated CD4+ T cells compared to the same cells in progressors [77]. Higher frequency in memory CD8+ CD73+ cells, a subtype involved in the HIV-specific CD8+ T-cell responses, was observed in ECs compared to healthy controls and HIV+ patients, even for those on ART [78]. CD8+ T cells from ECs produced more CD107a, a marker of CD8+ T-cell degranulation following stimulation in response to HIV, compared with NC on ART [79]. Also, CD8+ T cells from ECs and VCs released more inflammatory cytokines and chemokines than NC. These soluble factors included tumor necrosis factor-alpha (TNF- $\alpha$ ) and MIP-1 $\beta$ , which facilitate cytotoxic T cell lysis of HIV-infected cells [79,80]. Inhibiting the function of chemokine ligands *in vitro* led to loss of viral control and replication of HIV in susceptible T lymphocytes [81]. This may serve as one method by which ECs are able to achieve viral control.

Other studies performed in CD4+ T cells isolated from ECs have been aimed at understanding how these individuals are able to control viral replication. CD4+ T cells from ECs have been shown to retain their ability to proliferate and produce interleukin-2 (IL-2) in response to HIV [80,82,83]. Moreover, control of HIV replication

has been associated with high levels of HIV-specific interferon-gamma (IFN- $\gamma$ ) CD4+ T cells and lower levels of T-cell activation and HIV-neutralizing antibodies [82-84]. However, HIV-specific CD4+ T cell responses have been suggested not play a direct role in controlling viral replication, at least in non-human primates infected with simian immunodeficiency virus [85].

#### *CD4+ T Cell Phenotype and Susceptibility to HIV Infection*

Whether CD4+ T cells from ECs are intrinsically more resistant to HIV infection has also been investigated; these results have been very controversial, dependent on the method of CD4+ T cell stimulation. Polyclonal, PHA-activated ECs, and LTNP CD4+ T cells were susceptible to HIV infection [52].

In contrast, CD3-activated CD4+ T cells from ECs were resistant to HIV infection in culture, independent of co-receptor usage [86,87]. This phenotype was associated with increased levels of the cyclin dependent kinase (CDK) inhibitor p21 [86,88]. Further investigation of the role of p21 in ECs suggests that it may indirectly block HIV reverse transcription by inhibiting CDK2-dependent phosphorylation [89]. A recent study demonstrated that a subset of ECs have CD4+ T cells that produce higher levels of MIP chemokines, suggesting that these cells may be resistant to HIV infection by blocking R5-tropic HIV viral entry [90]. Conversely, HIV infection of CD3-activated CD4+ T cells from ECs and NCs was similar [91]. Non-activated CD4+ T cells from ECs were fully susceptible to HIV infection, similar to those of progressors. Higher levels of viral particle production, however, were observed in NCs compared with ECs [92,93]. Unstimu-

**Table 3: Summary of retrospective cohort studies of clinical outcomes in ECs.**

Study population	Sample size (N)	Primary outcome	Study period	Relevant results	Ref
HIV+ in the military healthcare system	Total (4,586) <ul style="list-style-type: none"> <li>• EC (25)</li> <li>• VC (153)</li> <li>• LTNP 10 (52)</li> <li>• LTNP 7 (101)</li> </ul>	Time to develop AIDS	1986-2006	<ol style="list-style-type: none"> <li>1. Time to virologic suppression was early after infection (less than 1 year from the time of sero-conversion) in most ECs/VCs</li> <li>2. ECs/VCs had fewer deaths and AIDS-defining events, and longer time to AIDS and death compared to NC</li> <li>3. Individuals achieving LTNP status for 10 years had more favorable time to AIDS and death compared to LTNP reaching their status for 7 years</li> </ol>	[43]
HIV research network	Total (34,000) <ul style="list-style-type: none"> <li>• EC (149)</li> <li>• High/ low VL (12,847/ 12,044)</li> <li>• NC (9,226)</li> </ul>	All-cause hospitalization rates	2005-2011	<ol style="list-style-type: none"> <li>1. ECs had higher rates of hospitalization rates due to CV disease and psychiatric illness, compared to NC under ART</li> <li>2. ECs were more likely to be hospitalized than VCs (with both high and low VL) due to CV diseases</li> </ol>	[135]
US military HIV+ natural history	Total (1091) <ul style="list-style-type: none"> <li>• EC (33)</li> <li>• VC (188)</li> <li>• Progressors on ART (870)</li> </ul>	Non-AIDS	2000-2013	<ol style="list-style-type: none"> <li>1. Non-AIDS infection was the most common reason for hospitalizations in all groups, ECs, VCs and progressors on therapy</li> <li>2. No differences in hospitalization rates associated with CV disease between groups, suggesting longer follow up of patients may be needed</li> </ol>	[134]
HIV+ patients from a University Hospital	Total (574) <ul style="list-style-type: none"> <li>• EC (64)</li> <li>• VC (76)</li> <li>• NC (434)</li> </ul>	Non-AIDS and AIDS events	1996-2011	<ol style="list-style-type: none"> <li>1. Non-AIDS-defining malignancies were the most common reason for hospitalization, followed by CV and neuropsychiatric illnesses</li> <li>2. The risk of non-AIDS events was comparable in ECs, VCs and NCs</li> <li>3. Only controllers who retained spontaneous control during the entire follow-up period had a lower risk of non-AIDS events</li> </ol>	[152]

EC: elite controller; VC: viremic controller; LTNP: long-term non-progressor; LTNP 7: LTNP through 7 years of follow-up; LTNP 10: LTNP through 10 years of follow-up; NC: non-controller; ART: antiretroviral therapy; CV: cardiovascular; VL: viral load; AIDS: acquired immune deficiency syndrome

lated CD4+ T cells from ECs exhibited reduced levels of viral integration, compared to those of NCs and HIV-negative controls [94].

On the other hand, ECs do not exhibit some of the immune changes that are observed in NCs. Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) is upregulated on HIV-specific CD4+ T cells during acute HIV infection, and also correlates with progression of disease. However, this phenotype has not been observed on CD4+ T cells from ECs [95,96]. Interestingly, ECs harbor lower levels of integrated HIV DNA, but higher levels of 2-LTR circu-

lar HIV DNA, suggesting a block at genome integration, after nuclear entry [97].

Other groups have studied whether the cellular phenotypes observed in natural killer (NK) cells were associated with ECs phenotype [98]. Undetectable viremia observed in ECs was shown to correlate with a higher percentage of activated NK cells [99]. Also, it has been suggested an increased NK activity in ECs who lacks HIV-1 specific CD8+ T cell responses [100]. Recently, the maintenance of CD4+ T cells in ECs has been associated with the lack of expression of one of the natural



cytotoxic receptors in NK cells [101].

### Host Genetic Factors

Varied approaches have been taken to identify potential host factors and genes involved in virologic control in both ECs and LTNPs (see Table 2). Several alleles within the HLA-B/C haplotype block have been associated with control, including HLA-B\*5701, HLA-C, and HCP5 alleles [39,102-109]. Furthermore, the presence of the CCR5 delta 32 ( $\Delta 32$ ) allele (a 32 base-pair deletion in CCR5 which renders the co-receptor cytosolic and non-functional) confers protection against seroconversion, with homozygotes being completely resistant to infection by R5-tropic viral strains [110,111]. HIV+ individuals who are heterozygous for the  $\Delta 32$  CCR5 genotype have relatively normal levels of CD4 T cell surface CCR5 expression but delayed disease progression [112]. Specific alleles of zinc ribbon domain containing (*ZNRD1*, a subunit of RNA polymerase I) and ring finger protein 39 (*RNF39*, a poorly characterized gene) were associated with progression [105].

Genome-wide association studies (GWAS) of HIV-infected cohorts evaluated associations between naturally occurring single nucleotide polymorphisms (SNPs) and particular phenotypes of interest (Table 2). In examining thousands of ECs and NC, the International HIV Controllers Study identified over 300 SNPs located within the chromosome 6 significantly associated with HIV control [51]. Specific amino acid sequences identified within the HLA-B peptide-binding groove were shown to have extremely low *P* values, lower than any other SNP found by GWAS, or any other HLA allele [113]. Imputed amino acids within the HLA-B peptide-binding groove, in addition to an independent HLA-C effect, explained the associations and the risk and protective alleles, suggesting that very specific interactions between HLA and viral peptides contribute to viral control. In particular, B\*57:01, B\*27:05, B\*14/Cw08:02, B\*52 and A\*25 alleles were protective, whereas B\*35 and C\*w07 conferred risk. Importantly, however, only ~20 percent of the protective effect was explained by the identified SNPs [51], suggesting that other, unknown genes and mechanisms are responsible for the observed control.

Additionally, it has been reported that TRIM-5 $\alpha$  expression contributes to viral control in EC patients expressing HLA-B\*57 or HLA-B\*27 alleles [114].

Investigators have also focused on the role of genetic and molecular factors, including those that regulate chromatin and DNA methylation, in viral control. Epigenetic modifications of the HIV promoter have been associated with control of HIV replication and transcription. ECs were shown to have higher levels of DNA methylation in the 5'-LTR compared with progressors [115]. Similarly, lower levels of *ccr5* gene DNA methylation were seen

in EC and HIV suppressors compared with HIV-negative individuals, indicating an association between *ccr5* methylation status and HIV disease [116]. DNA demethylation of regions that regulate PD-1 gene expression in HIV-specific CD8+ T cells was also associated with HIV control, in both ECs and NC on ART [117].

### CLINICAL OUTCOMES OF CONTROLLERS VS. NON-CONTROLLERS ON ART

The long-term clinical outcomes of ECs, as compared to NCs, have been mainly focused on progression to AIDS and AIDS-related death [43]. More is now known about the non-AIDS related clinical outcomes of ECs and the role that chronic immune activation plays in their outcome.

Several retrospective studies have tried to better understand the potential benefit of early ART in modifying both AIDS-related and non-AIDS related outcomes in controllers [118]. A summary of these studies examining clinical outcomes of ECs is provided below (Table 3).

#### *AIDS-associated Clinical Outcomes*

CD4+ count is the most well-recognized and reliable clinical indicator of HIV disease progression. For many years CD4+ number was paramount in treatment guidelines regarding timing of ART initiation [119-121].

Although early, high HIV RNA levels have been associated with CD4 decline [120,122], it is subsequent or set point viral RNA levels that have a greater prognostic impact on disease progression [123]. ECs achieve lower early baseline and set point viral RNA levels compared to NCs, and therefore have lower rates of AIDS progression and associated mortality [44,45,120,122] (Figure 3).

In a retrospective study, Okulicz et al [43] showed that among most ECs/VCs, virologic suppression occurred early after infection, and in most cases, during the first year from the time of known seroconversion. However, they did uncover differences between ECs and VCs, including more stable and higher CD4 counts in ECs than VCs. They also evaluated the time to AIDS and death among LTNP through 7 years of follow-up and 10 years of follow-up, depending on the duration of non-progression. Results showed that individuals achieving LTNP status for 10 years had more favorable time to AIDS and death compared to those achieving LTNP status earlier. Eventually, however, some VCs did progress to AIDS and death, reaffirming the notion that loss of virologic control and immune function occurs in some of these individuals. In fact, a study of more than four hundred ECs revealed that almost 30 percent of them lost viral control, resulting in reduced CD4 counts, underscoring the concept that many of these patients may eventually progress to AIDS [124].

### Non AIDS-associated Clinical Outcomes

ECs and VCs have higher levels of circulating inflammatory cytokines and rates of coronary atherosclerosis compared to NC on ART [125,126], suggesting that the chronic inflammation present may account for early vascular dysfunction [127]. Several factors contribute to the heightened inflammatory cellular setting of ECs and VCs compared to NC on ART, including gut microbial translocation and chronic T cell activation [128,129]. Gut microbial translocation is measured as circulating lipopolysaccharide (LPS) levels, and its presence has been attributed to viremia and disease progression [13,130,131]. Thus, it has been clearly demonstrated that ART reduces LPS levels and reduces the degree of immune activation [132,133]. The inflammatory environment that may be responsible for maintaining strict virological control in ECs and VCs may also portend unfavorable long-term clinical outcomes. A recent retrospective study revealed that non-AIDS-defining infections were the most common reason for hospitalization in ECs, with the same rates of hospitalization due to CV disease in both progressors on ART and ECs [134]. Crowell and colleagues [135] showed, however, that compared to NCs on ART, ECs had higher rates of all-cause hospitalizations due to CV disease and psychiatric illness. In light of what is known about the association of inflammatory cytokines and coronary artery disease in controllers, perhaps this finding is not surprising. That VCs had more favorable all-cause hospitalization rates due to CV disease compared to ECs was nonetheless unanticipated and certainly counter-intuitive.

In light of these findings it was of interest to determine the clinical outcomes of ECs and VCs after beginning ART. A recent study demonstrated an increase in CD4 number after ART initiation in both ECs and VCs, although it was somewhat better in the former [136]. Treatment with ART in ECs and VCs for six months reduced levels of immune activation markers and HIV VL (the latter in VCs), indicating that the use of ART in this setting may be beneficial [137]. Whether there are long-term, meaningful, and lasting differences in clinical outcomes remains to be established. Per current DHHS guidelines, starting ECs on ART is an individualized decision; all VCs should be on ART given their higher, detectable VLs.

### CONCLUSIONS

ECs and VCs are able to achieve spontaneous control of viral replication to differing degrees, in the absence of antiretroviral medications. This relatively rare ability is thought to be mediated via either viral or host immune or genetic factors. HIV-specific immune activation, a greater poly-functional CD8+ T cell response,

and HIV-specific CD4+ T cell responses in ECs may indeed play a significant role in reducing VL and delaying disease progression. Also, ECs have SNPs within the HLA loci that are significantly associated with viral control and finely map to the peptide-binding groove of the class I molecule. Functional and biochemical studies, however, are required to confirm the role of these amino acid residues in virologic suppression. Additional studies are necessary to pinpoint novel pathways and causal host genes responsible for virologic control, especially since the SNPs observed in the HLA loci can only explain ~20 percent of the EC phenotype. A better understanding of the mechanisms that underlie virologic control and the long-term clinical outcomes of ECs/VCs may help inform the 'HIV cure' agenda and lead to a better quality of life, even for HIV+ progressors.

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