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SUPPLEMENT ARTICLE

Clonal Expansion of Human Immunodeficiency Virus–Infected Cells and Human Immunodeficiency Virus Persistence During Antiretroviral Therapy

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The latent HIV-1 reservoir in blood decays very slowly, even during prolonged suppression of viral replication by antiretroviral therapy (ART). Mechanisms for reservoir persistence include replenishment through low-level viral replication, longevity and homeostatic proliferation of memory T cells, and most recently appreciated, clonal expansion of HIV-infected cells. Clonally expanded cells make up a large and increasing fraction of the residual infected cell population on ART, and insertion of HIV proviruses into certain host cellular genes has been associated with this proliferation. That the vast majority of proviruses are defective clouds our assessment of the degree to which clonally expanded cells harbor infectious viruses, and thus the extent to which they contribute to reservoirs relevant to curing infection. This review summarizes past studies that have defined our current understanding and the gaps in our knowledge of the mechanisms by which proviral integration and clonal expansion sustain the HIV reservoir.

Keywords. HIV infected cell proliferation; clonal proliferation; integration sites; cancer genes; T-regulatory cells.

The potential for curing human immunodeficiency virus (HIV) infection is limited by an incomplete understanding of the mechanisms that allow HIV to persist during antiretroviral therapy (ART). In the 1990s, ART was predicted to completely inhibit HIV replication and, based on the decay of cell-free and cell-associated HIV in the blood, to result in clearance of HIV infection within approximately 3 years [1]. However, cells capable of producing infectious viruses were found to persist, mostly composed of memory CD4 T cells [2–7] with a half-life of 4.6–44 months [8, 9]. Then, long cellular lifespans and/or low levels of replication were suspected to sustain viral reservoirs [8]. More recently, homeostatic proliferation, central to survival of mature CD4 T cells [10] and proliferation of HIV-infected cells [11, 12] was also hypothesized to sustain infectious reservoirs. The finding that certain tissues have low, perhaps subinhibitory concentrations of antiretroviral drugs (ARVs), lent support to the concept of ongoing replication within certain tissues during ART [7, 13, 14]. However, analyses of viral sequences from many individuals with ART suppression for more than a decade have provided no evidence of virus evolution or selection of drug-resistant variants [15, 16]. This review will focus on the most recently recognized mechanism for sustaining virus reservoirs, that of infected cell proliferation.

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PROLIFERATION OF HIV-INFECTED CELLS UNDER ART

Although approximately 75% of HIV infections result in the emergence of a single founder virus, and thus a nearly homogeneous virus population [17–21], stochastic as well as immune escape-driven viral evolution quickly results in virus diversification. Within a period of months, few identical viral sequences are found within more variable regions of the viral genome [22–25]. It was therefore surprising to observe that studies aimed at detecting "occult" HIV replication during ART [11, 12, 26] found that low-level viremias, between 50 and 250 c/mL during ART, were often composed of virions with identical *env* and *pol* sequences [12]. Often, these viral sequences were also identical to proviruses within infected cell DNA, leading to the hypothesis that clonal outgrowth of long-lived HIV-infected cells was occurring and that these cells could produce low levels of monotypic viremia [11, 12, 27]. The levels of viremia observed suggest that multiple cells from a clonal lineage were synchronously producing virions, possibly due to antigen-specific activation of memory cells, because single cells would not be capable of producing these quantities of virions.

The substantial proliferative potential of memory $CD4^+$ T cells [28] and the demonstration that clonal viral sequences are found within these cells [7] led us to hypothesize that proliferating cells would form an increasing fraction of the residual HIV-infected population under ART [29]. This would not be the case during typical untreated infections because newly infected cells would be much more numerous than the persisting infected cell population. This would explain why despite many years of ART, the level of infected cells in the blood and the amount of infectious

virus induced by global activators such as phytohemagglutinin (PHA) decline very slowly [8]. Our analysis [29] of 14 individuals over a median of 9.8 years of ART found that the proportion of all cell-associated viral DNA sequences corresponding to demonstrably clonal sequences increased at an average rate of 3.3% per year. Overall, infected cell numbers decreased over the study period, but the population of nonidentical viral sequences decreased by an average of 6.2% per year (95% confidence interval [CI] = 3.0–9.5%; $P < .0001$), whereas the clonal cell lineages experienced little if any decrease (mean = −1.0% per year; 95% CI = −0.2% to 2.3%; $P > .10$). Hence, although the number of infected cells decreases slowly under ART, the complexity of the virus population decreases more rapidly due to the increasing representation of what appeared to be clonally proliferating cells.

The aforementioned studies characterized viruses as "identical" using only short regions of the viral genome, usually 5%–10% of the entire virus, and in some cases, relatively highly conserved genes such as *pol*. In theory, if ARV levels are subtherapeutic during ART, an infected cell could transmit nearly identical virions cell-free, or more effectively through cell-to-cell contact, to vast numbers of susceptible cells. Thus, because analyses of short regions of HIV can overestimate the number of viruses thought to be emerging from proliferating cells [30], methods identifying HIV integration sites (HIV-ISs) are likely to be more accurate in identifying the source of monotypic virus populations.

To formally prove that identical viral sequences were due to infected cell proliferation, we developed the integration site looping assay (ISLA), which amplifies a region of the viral genome that includes the variable regions within the gp120 Env coding sequence, the genomic region with the highest genetic diversity, through the 3' long terminal repeat (LTR) and into the human cellular genome sequence flanking the viral integration site [31] (Figure 1). The utility of this approach stems from the extremely large number of possible HIV-ISs in the human genome and the extremely low likelihood that any 2 sites would be identical, as discussed below. Using the ISLA, we initially identified the host sequence at HIV-ISs plus the 3' end of the viral genome (Figure 1, sections 1–4). Using primers specific to this host sequence, the viral sequences of the adjacent 2.8-kb region encompassing a portion of *env*-gp120, *nef*, and the remainder of the LTR were then recovered and sequenced (Figure 1, section 5) [31]. Using this method, each IS was linked to clonal provirus populations previously identified and to plasma HIV sequences. These studies provided a formal proof that identical proviral sequences arise from the proliferation of infected cells. Furthermore, in all cases evaluated to date (eg, Mullins JI, Hall B, Kim K, Deng W, Westfall DH, Bull M, Chen L, Aamer HA, Frenkel LM. 2015. Integration Sites & Propagation and Analysis of Individual Lineages of HIV-Infected Cells Persisting during ART. Banbury conference on HIV-1 and How to Kill a Killer: Attempts at Total or Functional Cure of HIV-1, Cold Spring Harbor Laboratory, October, 2015.) identical viral sequences were found to match the same ISs. And, identical whole genome sequences were obtained from several proviruses with identical ISs (James I. Mullins, Malika Aid, Hadega A. Aamer, Paul Edlefsen, Sherry McLaughlin, Marta E. Bull, Hannah Huang, Sheila Styrchak, Wenjie Deng, Thomas Sibley, Evan Silberman, Rafick Sekaly, and Lisa M. Frenkel, manuscript in preparation).

Ikeda and colleagues first demonstrated multiple identical HIV-ISs in longitudinal samples from individuals on ART, which suggested clonal expansion and long-term persistence of infected cell clones [32]. Subsequently, 2 studies firmly established the pervasiveness of clonal expansion, showing that such cells can make up a large fraction of the residual HIV population in individuals on ART. Using a high-throughput deep-sequencing approach to identify HIV-host sequence junction fragments, Maldarelli and colleagues [33] evaluated 2410 ISs in 5 individuals and found clonally expanded cells to comprise approximately 40% of all HIV-ISs. This approach has a higher throughput than the ISLA but does not permit linkage to variable segments of the viral genome to demonstrate the origin of monotypic viral sequences and identifies ISs at both ends of the provirus without being able to distinguish whether different sequences observed derive from the same or different cells. Using the ISLA, we [31] identified 534 ISs in 3 individuals over 11–13 years of infection. This longitudinal study confirmed that infected cell clones persisted and, importantly, showed an overall increase in representation during ART [31]. Subsequently, Cohn et al [34] used a deep sequencing approach that inferred identical integrations from unique sheared ends of sequences. They tallied 6719 HIV-ISs from 5 individuals before and after starting ART, as well as from 4 untreated viremic individuals and 4 viremic controllers (viral loads ranged 430–1070 copies of HIV RNA/mL plasma). As with our results, they found the representation of infected cell clones to increase with time on ART. However, their results also departed in multiple respects from prior studies. They found clonally expanded cells at high levels in all individuals irrespective of viremia, an unexpected finding given that identical HIV *env* sequences were rarely detected in the vast number of prior studies of untreated noncontroller individuals in chronic infection. They also reported 78%–90% of all infected cells as being derived from proliferating cells, versus 15%–65% in Wagner [31] and McLaughlin et al. [33], despite a longer period on ART, and ~40% in the Maldarelli study.

DISTRIBUTION OF INFECTED PROLIFERATING CELLS IN TISSUES

Bull et al [35] demonstrated that infected cell clones populate the blood and the female genital tract, and we have found identical HIV-ISs in >12 tissues examined from multiple individuals at autopsy, indicating that clones of infected cells spread throughout the body (Mullins et al, manuscript in preparation). Cohn [34], as well as Boritz et al [36], while studying viremic

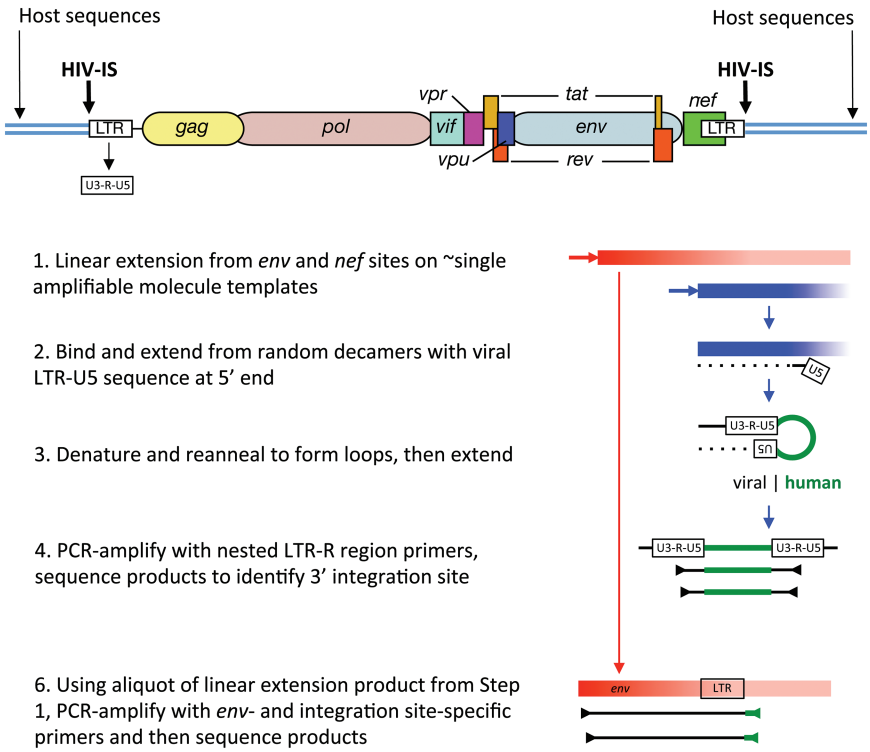

Figure 1. Schematic summary of the Integration Site Looping Assay (ISLA). Essential aspects of this procedure are schematically shown here, whereas full details are provided in Supplementary Figure 1 from [31]. The structure of a full-length provirus and adjacent cellular sequences are shown at the top of the figure. Long terminal repeated sequences (LTRs) flank the provirus, with LTRs subdivided into U3, R, and U5 segments. These segment names derive from their origins in viral genomic RNA; they are found to be: unique to the 3′ end of the human immunodeficiency virus (HIV) genomic RNA (U3), repeated at each end of the HIV genomic RNA (R), and unique to the 5′ end of the HIV genomic RNA (U5). Using 2 virus-specific primers from the *env* and *nef* genes, linear amplification is carried out to increase the representation of these sequences in the cellular DNA preparation (Step 1). Products extending from the *nef* primer that reach the adjacent host sequences are much shorter (approximately 0.8kb vs approximately 2.8 kb) and hence are much more efficiently generated. An aliquot of this reaction is then used to extend DNA from random primer sequences containing the U5 region at the 5′ end (Step 2). This product is then denatured and reannealed to form a loop with the U5 region of the provirus hybridizing to its complement derived from extension products generated with random primers (Step 3). Such products are then selectively polymerase chain reaction (PCR)–amplified using R region primers and then sequenced to determine the host genome sequences immediately adjacent to the 3′ end of the provirus (ie, the integration site, Step 4). To link the integration site to a specific proviral genome, the sequence obtained needs to extend into the gp120 protein coding sequence of the viral *env* gene, the region of highest level of viral genetic variation. Hence, in step 5, integration site (IS)–specific primers are generated to provide the specificity to amplify the longer, less efficiently produced *env* IS products.

controllers, found infected cell clones in Central memory T-cells (T_{CM}), Transitional memory T-cells (T_{TM}), and Effector memory T-cells (T_{EM})memory cell types. Boritz also found the same HIV-ISs in cells of multiple memory cell types, indicating that these cells were infected before undergoing differentiation into T_{EM} cells.

INFECTED PROLIFERATING CELLS HARBOR DEFECTIVE AS WELL AS INFECTIOUS PROVIRUSES

The vast majority of infected cells that persist under ART harbor defective proviruses [37–42]. However, such proviruses are not necessarily innocuous: (1) Defective proviruses, including those from infected cell clones, are capable of producing viral antigens and viremias and populate tissues throughout the body [43–46]. This chronic antigenic stimulation could contribute to the inflammatory pathology observed during ART (Richard Fox, Brian Johnson, Atis Muehlenbachs, Paul T. Edlefsen, Dylan Westfall, Julie Elliott, Peter Anton, Lisa M. Frenkel and James I. Mullins, submitted). (2) Defective proviruses may undergo spontaneous partial repair in vivo [47], and immunodeficiency disease can develop following spontaneous repair in vivo of *nef*-deleted simian immunodeficiency virus (SIV) in macaques [48]. Data from the Sydney Blood Bank Cohort have shown some evidence of disease progression in people infected with viruses harboring defective *nef* genes [49–51]. (3) Infectious viruses can be rescued by recombination between 2 defective proviruses [52, 53] and following superinfection [54].

Clonally proliferating cells have been hypothesized to persist due to their genomes being noninfectious [31, 33, 34]. However, Anderson and colleagues have found that clonally produced plasma RNA can be recovered as virus from resting $CD4^+$ T cells [55]. Simonetti, Bull, and their colleagues have separately shown that clonally proliferating cells can contain intact full-length proviruses and produce infectious viral genomes [44, 56]. Because >90% of proviruses examined under ART have been found to be defective [41, 42] (Breana M. Hall, Noelle

P. Dahl, Katie B. Kim, Lennie Chen, Michael P. Dapp, Anne-Marie Rousseau, Tamara Babenko, Dylan H. Westfall, Hannah Huang, S. Styrchak, K. Wong, H. Zhao, Thomas R. Sibley, Evan J. Silberman, C. Alexander Hill, L.M. Frenkel and J.I. Mullins, manuscript in preparation), the extent to which clonally proliferating cells contribute to the residual infectious reservoir remains to be quantified.

FAVORED SITES OF HUMAN IMMUNODEFICIENCY VIRUS INTEGRATION

Integration of the HIV proviral genome involves duplication of a 4–5-basepair region of the host cellular DNA adjacent to the LTR at each end of the integrated viral genome [57]. The site at which the virus integrates into the host genome is not precise. If it were, we could not use identical integration sites to quantify the proliferation of infected cells. Pioneering and comprehensive studies by Bushman and colleagues [57, 58], as well as others [59], have established that there is no absolute chromosomal site or sequence specificity for virus integration within the host genome. Rather, HIV integration tends to favor repeated sequence elements in the host [34, 60, 61] and structural features such as bent or distorted DNA within nucleosomes [62, 63]. It is also clear that virus integration is favored within genes over intergenic regions of DNA in both T-cell and macrophage cultures and in vivo [23, 32, 64–67], especially within actively transcribed genes [64]. Thus, regions of open chromatin found during transcription, and potentially during cell division, serve as targets of HIV-ISs. Interactions between viral integration complexes and cellular proteins such as LEDGF/p75 enhance viral integration [68, 69] and tether viral preintegration complexes to specific regions [70, 71]. In addition, the close proximity of a gene to nuclear matrix attachment sequences [72, 73] and the nuclear periphery [74], especially nuclear pores [75], are also associated with increased frequencies of HIV-ISs, with depletion of nuclear pore proteins diminishing HIV-ISs in gene-dense regions [76, 77]. Lastly, transcription-associated histone and DNA modifications [78] are associated with increased HIV-IS frequencies. Interestingly, Cohn [34] reported that ART was associated with a decrease in cells with HIV-ISs in highly expressed genes.

GENES ASSOCIATED WITH HUMAN IMMUNODEFICIENCY VIRUS INTEGRATION SITES AND CLONAL PROLIFERATION

Our comparison of the patterns of HIV-ISs from individuals on suppressive ART to >44 000 HIV-ISs found after acute infection of Jurkat or primary cells in vitro [78, 79] showed strong clustering of HIV-ISs in vivo but far less so in vitro and little evidence of clonal proliferation in vitro [31]. Similar results were found by Maldarelli and colleagues using their own cell culture HIV-IS data [33, 80]. Clonal proliferation would not necessarily be expected to be observed in recently infected cells in culture because of the limited time permitted for selective cell outgrowth. In contrast, the dramatic clonal cell proliferation observed in ART-treated individuals and the clustering of sites in certain genes, strongly suggests that these phenomena occur as a result of a selective process. Hence, we examined the distribution of HIV-ISs across genes [31]. Genes associated with cancer were substantially overrepresented among HIV-ISs during ART ($P < .0001$), but this bias was also observed in the HIV-ISs from in vitro infections (12.70% vs 11.14% of sites, respectively). Cells undergoing clonal proliferation, however, had an excess of HIV-ISs in genes associated with cancer ($P = .008$) and above that found for HIV-ISs in cell culture ($P = .049$). This cancer gene association was derived using gene sets compiled from several sources [31]. When gene ontology (GO) terms were used instead, uniquely detected HIV-ISs and HIV-ISs in infected cell clones were enriched in genes with hallmark cancer GO terms, as well those for cell proliferation and negative regulation of cell cycle (all with $P < .0001$). These data strongly support the hypothesis that HIV integration can promote cell proliferation and survival.

Ikeda and colleagues were also first to show clusters of HIV-ISs in a particular gene, the basic leucine zipper transcription factor 2 (*BACH2*), which they found in 2 individuals [32]. Human immunodeficiency virus integration sites have been found in this gene in a total of 9 of 33 (27%) individuals at 72 unique sites in the host, including 24 that have been shown to be from infected cell clonal populations [31–33, 41, 65, 81, 82] (the Cohn et al study [34] was not included because no precise HIV-IS mapping data was available at this writing). As previously noted [31], a striking feature of the HIV-ISs in *BACH2* is that all are found in the same transcription orientation as the gene and in the 2 introns just upstream of the start of the coding sequence. It is also notable that integrations of murine leukemia virus into *BACH2* have been associated with B-cell tumors [83]. *BACH2* has been linked to a large number of functions in humans (eg, autoimmune and allergic diseases; activation of the tumor suppressor p53; regulation of a variety of immune cell functions, including CD4 T-cell senescence; immune activation; regulation of naive cells; generation of T_{EM} cells; and formation and regulation of T regulatory cell (T_{reg}) function [84–89]). It is therefore quite conceivable that *BACH2* plays a role in HIV-infected cell survival and proliferation.

BACH2 is by no means the only gene selected for persistence and proliferation in association with HIV integration. Human immunodeficiency virus integration sites in the MKL1/myocardin-like-2 (*MKL2*; also referred to as megakaryoblastic leukemia 2) gene have been found in 4 individuals, at 21 different sites, including 11 in clonally proliferating cells [31–33]. Like *BACH2*, *MKL2* is a nucleic acid binding protein and is known to be a transcription coactivator of serum response factor (which regulates cell growth) and has been associated with lipomas [90] and perhaps cancers [91]. Human immunodeficiency virus

integration sites were also found in the same transcriptional orientation as the *MKL2* gene at 20 of 21 sites. Human immunodeficiency virus integration sites within the related *MKL1* gene has been found in 2 individuals, with HIV-ISs at total of 5 sites, including 2 in proliferating infected cell clones [31, 33].

Although many other genes are worth mentioning, the last we will discuss here is the signal transducer and transcription activator in JAK/STAT pathway 5B (*STAT5B*). This gene is involved in autoimmunity [92] and T_{reg} maintenance [92, 93], T-cell receptor signaling, apoptosis, and different isoforms of the gene are associated with different cancers (eg, [94–97]). Human immunodeficiency virus integration sites in this gene have been found in 10 individuals, at 19 different sites, including 5 in clonally proliferating cells [31–33]. Unlike *BACH2* and *MKL2*, however, no strong directional bias was observed in these ISs, with 12 of 19 in the same transcriptional orientation as the gene.

LATENCY AND THE PAUCITY OF T-CELL TUMORS

The site of virus integration has a strong influence on the level of expression of the provirus, and thus the development of latency, evident in both cell culture infections and HIV-ISs from patient specimens (ie, in vivo) [65, 98, 99]. Although HIV-ISs are preferentially found in highly expressed genes, elegant studies by Verdin and colleagues have shown that latency is readily induced after infection of cell lines and that the transcriptional activity of proviruses overall is inversely correlated with the transcriptional level of the gene [98–100] and is particularly low in proviruses found in heterochromatin [99] and gene deserts within euchromatin [100]. Furthermore, latency can be similarly and rapidly established after ex vivo infection of resting and activated human $CD4^+$ T cells as well as tonsil and spleen cells [101]. DNA modifications such as cytosine methylation, histone methylation, and acetylation modifications also affect gene expression. Enzymes that modulate these modifications have become important targets of research and clinical studies directed to reversing viral latency (discussed in detail by Margolis in this supplement).

Human immunodeficiency virus may rarely cause insertional mutagenesis [102, 103], resulting in the development of tumors from infected cells in a few cases [80, 104, 105]. Notably, the infectious provirus found in clonally expanded cells reported by Simonetti was found in a person with a squamous cell cancer [106], and a prior report demonstrated clonality of HIV-ISs in association with a Kaposi's sarcoma [107]. However, evidence for direct causation is lacking in the latter cases, as the virus was not shown to be in tumor cells. Kaposi's sarcomas (KSs) and non-Hodgkin's lymphomas accounted for approximately 90% of HIV-associated malignancies in the pre-ART era [108], and despite a sharp decline since the advent of effective ART [109], their attack rate remains much higher in HIV-infected individuals compared with the general population [110].

In contrast with HIV, infection with avian sarcoma/leukosis virus and murine (MLV) and feline leukemia viruses induce tumors within target cells by insertional mutagenesis [111] at relatively high frequencies. As noted herein, MLV integration within the *BACH2* gene leads to B cell lymphomas in mice [83]. There are at least 4 potentially important differences between the host–virus interactions of these animal retroviruses and HIV that may explain differences in virally induced tumor frequencies: (1) HIV has a more complex genome structure and transcriptional control apparatus compared with the animal retroviruses (eg, avian, murine, feline). For example, HIV Tat is required for high-level transcription and Rev for transport of incompletely spliced mRNA to the cytoplasm for production of viral structural proteins. These regulatory features, plus the host's local genetic and chromatin environment, typically lead to low levels of HIV gene activity and to latency. In contrast, little is known about animal retrovirus latency. (2) Animal retroviruses have a broader host cell range than HIV, and the infected cell burden in vivo is typically orders of magnitude higher than in HIV infection (eg, [112, 113]). (3) Murine leukemia virus integrations are often facilitated by interaction between the viral integrase and cellular BET proteins [114] and are found in association with promoter sequence elements and transcription start sites [115, 116]. Hence, MLV integrations are more likely to result in gene activation compared with the more random placement of HIV-ISs within genes. One MLV IS, found in association with B cell lymphomas, activated an alternative promoter, resulting in the production of a new *BACH2* protein isoform [83]. Each of these host–virus interactions are likely to result in higher tumor frequencies in animals compared with HIV infections. (4) Lastly, MLV integrations that lead to *BACH2*-associated B-cell tumors and all HIV-ISs found to date in people have been found in the 2 introns just upstream of the start codon. However, all HIV-ISs were found in the same transcriptional orientation as the gene, whereas the overwhelming majority of the MLV-IS were in the opposite orientation. This suggests a differential effect on cellular gene and protein expression, although frequent HIV-ISs in other genes occur in both the reverse and same transcriptional orientations (eg, in *STAT5B*) within proliferating cells.

These observations, along with the finding that infected cell proliferation occurs with HIV-ISs in genes associated with immune control such as T_{regs}, suggest the possibility that clonally expanded HIV-infected cells may contribute to an increased susceptibility to cancers by contributing to an immunosuppressive cytokine milieu [107] in which cancer cells are better able to develop and persist.

PROLIFERATION OF INFECTED CELLS AND OTHER OBSTACLES TO CURE

Infected cell clones clearly resist depletion during ART; however, we do not know to what degree these cells will resist

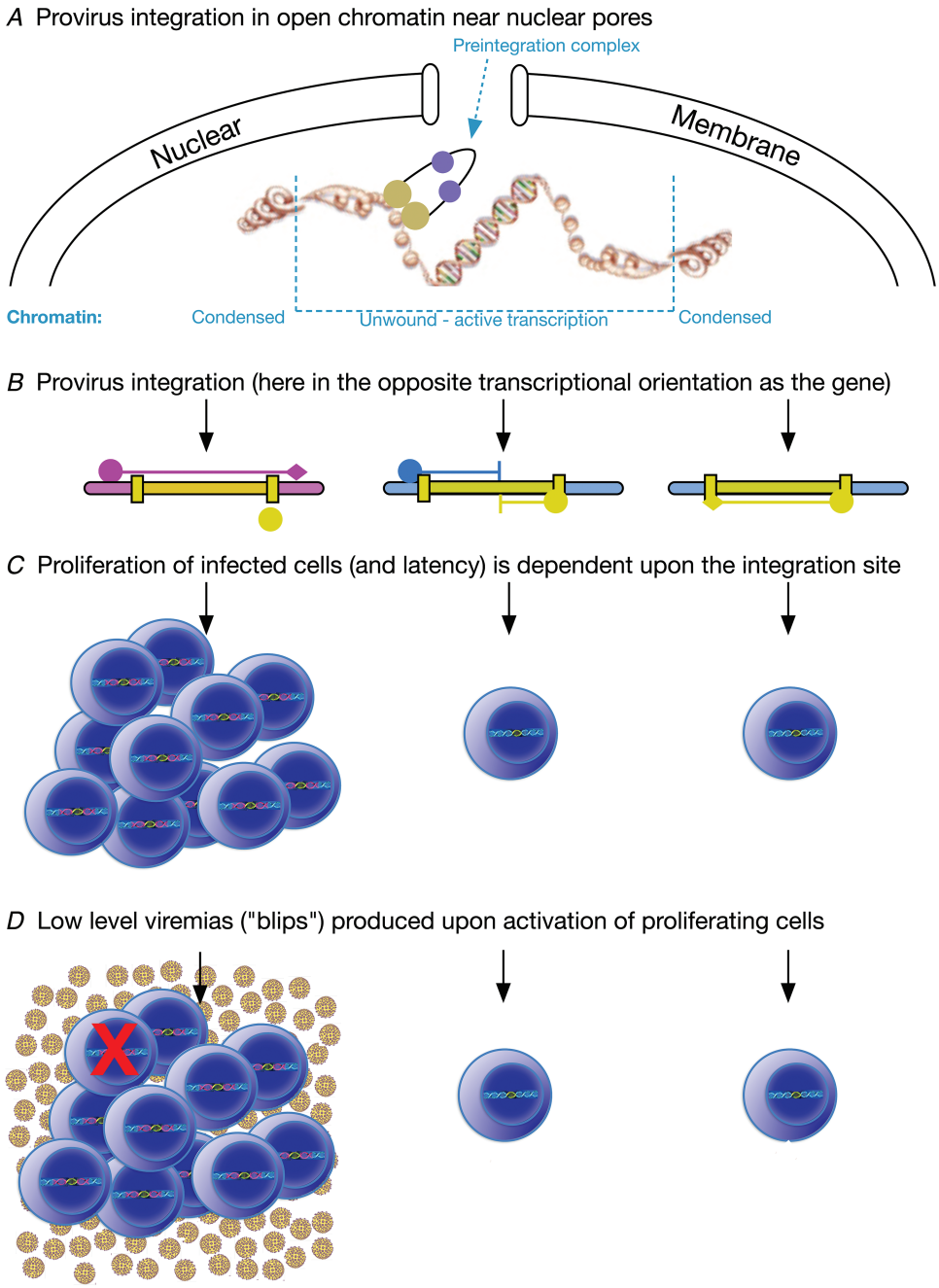

Figure 2. Summary of findings and hypotheses discussed in this review. *A,* Provirus integration is favored in transcriptionally active genes near the nuclear membrane and pores. *B,* Human immunodeficiency virus (HIV) integration can result in the provirus and gene being in the same, or as shown here, the opposite transcriptional orientation. Subsequently, several scenarios for host gene and provirus transcription are possible, with 3 shown: Left, host gene transcription is initiated (pink circle) upstream of the provirus and travels through the provirus then terminates downstream (pink diamond), interfering with transcription of the proviral 5′ LTR (yellow circle). Middle, transcription is initiated both within the cellular gene and the provirus, leading to a transcriptional collision and incomplete expression of both transcription units. Right, transcription within the provirus is initiated and terminated correctly, yielding virions. *C,* Proliferation of infected cells as well as viral latency is associated with specific integration sites. *D,* Low-level viremias can result from proliferating infected cell populations, most likely from synchronous activation of a specific clonal cell population, perhaps by its cognate antigen. An immune-suppressive milieu may develop by HIV integrations affecting the function of regulatory T cells (T_{reg}) cells that limit killing (red X) of virus-expressing cells, as well as cancerous cells (not depicted).

cure—as this will likely depend on the cure modality and the tissues in which the residual HIV reservoir is located. Although most studies of HIV reservoirs have focused on blood, it is highly likely that much more of the HIV reservoir is found in lymphoid tissues, the major site of viral replication early in infection [117, 118] when reservoirs are initially established [119, 120], or in organs with more pronounced physical barriers, such as the brain. Of particular interest

are HIV-infected CD4$^+$ T follicular helper (T_{FH}) cells, which migrate into B-cell follicles and promote B-cell maturation and antibody production [121]. T_{FH} cells within lymph nodes have been found to harbor a significant fraction of the (infectious) HIV reservoir under ART, and activated T_{FH} cells, in particular the PD-1$^+$CXCR3$^-$ subset, have been reported to make up a large fraction of the HIV reservoir in blood [122]. T follicular helper cells are also highly permissive for infection ex vivo and produce especially high levels of viral RNA found in lymph nodes [123, 124]. As will be discussed in more detail by Boritz and Douek in this supplement, a recent study of 14 natural HIV controllers (ie, <1000 copies of viral RNA/mL plasma) [36] showed that T_{EM} and T_{TM} cells in the blood were more likely to contain HIV DNA than T_{CM} cells and much more than naive T cells (T_N). However, when examining lymph nodes from 3 controllers, T_{FH} cells within and outside germinal centers had higher levels than T_{CM} and T_{EM} cells, all of which were higher than levels found in blood CD4 cells. Also notable was that plasma HIV RNA levels and genotypes were more closely related to viral genomes from the lymph node than to those in blood CD4 cells, arguing that lymph node was an important source of viremia in these individuals and/or that infected cells within lymph nodes were more likely to survive immune depletion.

The persistence of HIV-infected cells harboring infectious as well as noninfectious proviruses begs the question as to why these cells are not eliminated by the immune system once viral antigens are produced. Latency is one factor. In addition, HIV-infected cells may persist in anatomic and cellular sanctuaries that restrict infected cell-targeted immune responses. Fukazawa and colleagues showed that SIV-infected viral RNA-expressing cells are not cleared from lymph node B-cell follicles [125], suggesting that these follicles may be a "sanctuary" for SIV and, perhaps HIV. infection. This also helps explain why T_{FH} cells within lymph nodes harbor a significant fraction of the HIV reservoir under ART [36, 122, 126].

SUMMARY, CONCLUSIONS, AND SPECULATIONS

Human immunodeficiency virus integrates within the host genome preferentially in actively expressed genes and genes with spatial proximity to where the integration complex enters the nucleus and proximal to nuclear pores (Figure 2A). The site of viral integration in the host genome impacts viral latency. Although the mechanisms are unclear, integration into certain genes appears to favor persistence and proliferation of the cell and therefore is important to sustaining the HIV-infected cell population. Host gene transcription may interfere with viral transcription and vice versa (Figure 2B). Proliferating cells make up a substantial, if not a majority, component of the residual infected cell population under ART (Figure 2C) and have been shown to include infectious viruses in multiple studies. Because proliferating infected cells have memory phenotypes, cognate antigens may synchronously activate cell clones to produce the virions found in low-level viremias (or "blips") under ART (Figure 2D). Additionally, proliferating cells may exert an immunosuppressive effect due to the provirus affecting transcription of T_{reg} genes that impair their own elimination as well as elimination of other infected cells following activation (Figure 1D). Lastly, as individual cell purification methods are improved, proliferating infected cells, by virtue of their clonality, will provide a relatively rich source of material for study of the cellular environments that result in viral latency and recalcitrance to release from latency.

Notes

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