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Single-Cell Analysis Reveals Heterogeneity of Virus Infection, Pathogenicity, and Host Responses: HIV as a Pioneering Example

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Abstract

While analyses of cell populations provide averaged information about viral infections, single-cell analyses offer individual consideration, thereby revealing a broad spectrum of diversity as well as identifying extreme phenotypes that can be exploited to further understand the complex virus-host interplay. Single-cell technologies applied in the context of human immunodeficiency virus (HIV) infection proved to be valuable tools to help uncover specific biomarkers as well as novel candidate players in virus-host interactions. This review aims at providing an updated overview of single-cell analyses in the field of HIV and acquired knowledge on HIV infection, latency, and host response. Although HIV is a pioneering example, similar single-cell approaches have proven to be valuable for elucidating the behavior and virus-host interplay in a range of other viruses.

1. INTRODUCTION

Analyses of populations are very useful as they provide a general picture of the infectiousness of a virus or the features of a viral disease, including clinical signs and progression. However, population analyses average multiple individual infection outcomes that vary according to host genetic, environmental, and viral factors. Indeed, individuals are genetically unique, displaying specific responses to viral infections, from resistance to susceptibility toward acquisition, from low to high viremia, from long-term nonprogression to rapid progression of the disease, or from absence of control to elite immune control (**Figure 1*a***). Extreme phenotypes are thus usually masked in the average bulk population. In order to understand the genetic bases of these variable individual responses toward human immunodeficiency virus (HIV) infection, the past two decades have included a focus on genome-wide association studies highlighting a major role of human leukocyte antigens (HLAs) and C-C chemokine receptor type 5 (CCR5) loci (1–4). These studies allowed researchers to identify specific gene variants involved in HIV pathogenesis at large and explain a maximum of ∼30% of individual variation (5).

Similarly, individual cells also present variability toward HIV infection, despite mostly identical genetic content, except for HIV genome insertion, point mutations, or T cell receptor rearrangement (**Figure 1*b***). Therefore, infection success depends mostly on cell composition (with the spectrum of expressed gene products providing a favorable or restrictive environment for virus replication) and on the environment (extracellular milieu and interactions with neighboring cells). Improvement of technologies in the past decade has allowed for isolation and analysis at single-cell resolution, thereby providing tools to explore cell heterogeneity and identify gene products and cell biomarkers involved in specific HIV-related phenotypes (6). In this review we summarize the application of a wide range of such single-cell approaches and discuss examples of the integration of complementary results from different approaches. These results both illustrate powerful advances in understanding HIV replication and host interactions, and provide a useful example of the dramatic potential of emerging similar single-cell studies for other viruses.

2. SINGLE-CELL TECHNOLOGIES

Analyses at the single-cell level have traditionally used technologies with poor resolution, such as cell imaging or flow cytometry, providing limited information about the cell. The identification of the human genome sequence in the third millennium opened new perspectives to understand human and cell biology in a more comprehensive and genome-wide manner (7). Tang et al. (8) marked the second milestone and opened the way of single-cell technologies by publishing the first transcriptome-wide analysis of an individual cell by sequencing messenger RNAs (mRNAs) from a single mouse blastomer [single-cell RNA sequencing (scRNA-Seq)]. This analysis was the first step toward gathering a broader and more complete picture of the cell.

The study of individual cells is mainly a two-step process, requiring single-cell isolation on one hand and analysis of that cell at the molecular level on the other hand; these steps are briefly described here.

2.1. Single-Cell Isolation

The single-cell analysis workflow starts with sorting and isolating individual cells, which can be achieved using multiple approaches that diverge mainly in throughput and automation level (9–12). Limiting dilution of a cell suspension can be performed with conventional lab equipment; it is cost effective but labor intensive, resulting in a low cell throughput. Micromanipulation for manual cell picking or laser microdissection requires some specific equipment and results

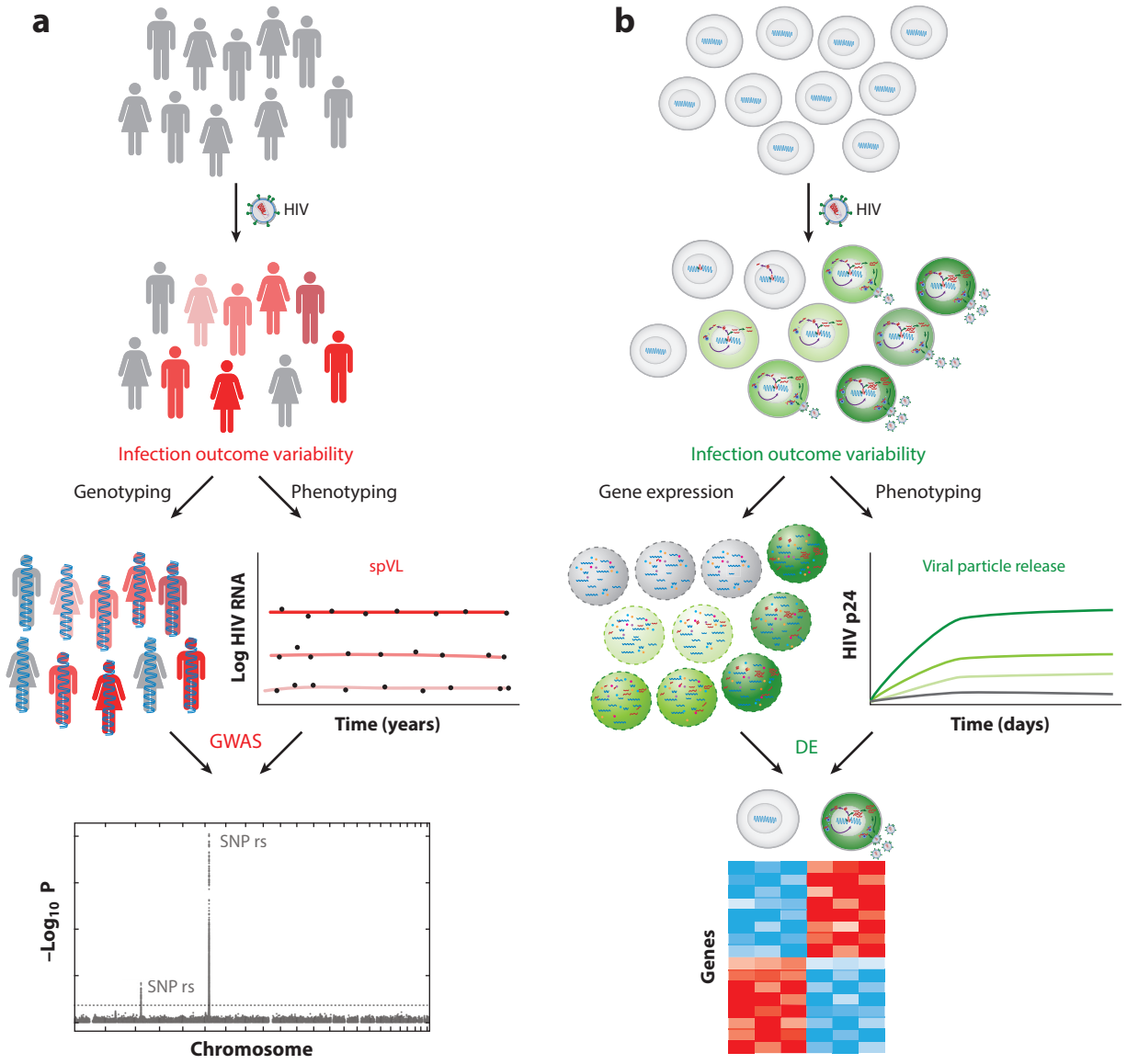


Figure 1

Exploring living heterogeneity as a tool to identify virus-host interactions. (*a*) Individuals are not equal toward HIV acquisition and HIV disease progression. Some people are resistant (*gray*), while other people are infected but are able to control viremia to undetectable or low levels, and hence they do not progress or progress slowly to disease (*light red*). In contrast, some people progress very rapidly to disease onset (*red*). Analysis of individual genotypes and correlation with a specific phenotype such as the spVL allow for GWASs and identification of gene variants that affect the phenotype outcome, either positively or negatively. (*b*) Individual cells are not equal toward HIV infection. Although almost genetically identical, cells from the same donor are not equally permissive to HIV infection, ranging from resistant cells (*gray*) to various levels of productive infection (*green shades*). Analysis of individual cellular content (RNA, protein, etc.) and correlation with a specific phenotype such as viral production allow for the identification of cellular proteins that affect the phenotypic outcome. Abbreviations: DE, differential expression; GWAS, genome-wide association study; HIV, human immunodeficiency virus; SNP rs, single-nucleotide polymorphism reference; spVL, set-point viral load.

usually in a low cell throughput. Fluorescence-activated cell sorting (FACS) allows single-cell sorting by flow cytometry using fluorescent reporters or antibody-based recognition of specific proteins. This approach can be coupled with automated single-cell dispensing, thereby increasing the cell throughput recovery. Although FACS can sort cells according to up to 18 colorimetric parameters, it requires pressurization to handle cells, which can induce damage. Novel devices have been recently developed, allowing automated single-cell sorting and dispensing based on a few parameters only but with lower pressure to minimize cell perturbation. Progress in microfluidics further allowed the development of well-based integrated circuits or droplet-based technologies to physically separate or encapsulate single cells, allowing for full automation and higher throughput. Further progress in nano- and picofluidics, combined with cell indexing and barcoding, further pushed forward the number of individual cells that can be isolated and analyzed. Thus, single-cell isolation techniques improved in the past decade, starting from one single cell isolated manually in 2009 to up to 100,000 cells isolated mechanically today (8, 11).

2.2. Single-Cell Analysis

Recent advances in molecular biology, such as sequencing technologies, allowed the development of methods supporting the study and characterization of individual cells. These methods include profiling of the genome (13–15), epigenome (16–18), transcriptome (6, 19–25), or proteome (26–28), and they can be used either alone or in combination (e.g., coupled analysis of RNA sequences with epigenetic modifications or protein abundance) (29–33) (**Figure 2**).

Although ideally single-cell analysis points toward integrated multi-omic analysis to gather the most complete picture of the single cell, the technology that is currently mostly used focuses on whole transcriptome analysis, as it can benefit from powerful high-throughput sequencing technologies and thus provide an extensive view of the RNA cell content (6, 11, 20, 29, 34, 35). In order to perform scRNA-Seq, the first barrier to overcome is the initial RNA material input. Indeed, a single cell contains between 1 and 50 pg RNA according to the cell type, which is not enough to perform RNA-Seq. To overcome this limitation, the RNA is first converted into complementary DNA (cDNA) and amplified before preparing sequencing libraries. Multiple methods exist for reverse transcription and cDNA library preparation, which differ mostly by three criteria: (*a*) full-length or 3′-end enrichment of RNA molecules such as switching mechanism at 5′ end of RNA template sequencing (Smart-Seq) and droplet-sequencing (Drop-Seq), respectively; (*b*) insertion or not of a unique molecular identifier (UMI) and a cell barcode; and (*c*) linear or exponential cDNA amplification followed by RNA-based library preparation or by tagmentation (reviewed in 11, 20, 21).

In a comparative study, Ziegenhain et al. (20) showed that full-length methods, i.e., covering the whole transcript but without UMIs or barcode, were more sensitive than multiplexed 3′-counting methods with UMIs. Indeed, using a cutoff of 1 million reads per cell, full-length based RNA-Seq allowed a median detection ranging from 7,572 to 9,138 genes, while the median detection of genes was generally lower for 3′-end based RNA-Seq samples, from 4,763 to 7,906 genes. Furthermore, pool analysis of 65 individual cells allowed detecting a total of ∼17,000 to ∼21,000 genes, similar to bulk RNA-Seq detection. Besides sensitivity, full-length methods are likely to be more appropriate for isoform analyses, alternative splicing, and single nucleotide polymorphism identification on a small size sample, while tag-based methods may be more adapted for gene expression quantification in a complex cell sample by allowing multiplexing and analysis of a larger number of cells at the same time.

Variability of gene expression across cells is very high, as 87% of genes are detected in only 1–2 single cells with a low level of expression (20). This single-cell transcriptome diversity has

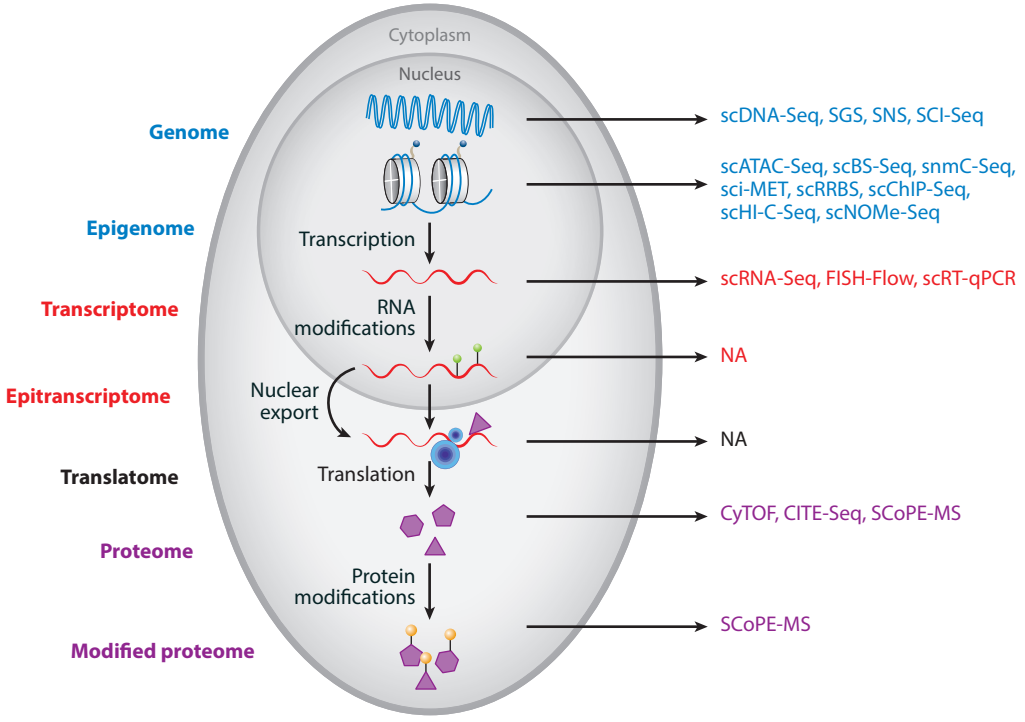


Figure 2

Single-cell technologies. Multiple single-cell methods have been developed in order to interrogate the various layers of the cell. The single-cell genome can be analyzed by single-cell DNA sequencing (scDNA-Seq), single-genome sequencing (SGS), short nascent DNA strand sequencing (SNS), and single-cell combinatorial indexed sequencing (SCI-Seq). The single-cell epigenome can be analyzed by single-cell assay for transposase-accessible chromatin followed by sequencing (scATAC-Seq), single-cell bisulfite conversion sequencing (scBS-Seq), single nucleus methylcytosine sequencing (snmC-Seq), single-cell combinatorial indexing for methylation analysis (sci-MET), single-cell reduced-representation bisulfite sequencing (scRRBS), single-cell chromatin immune-precipitation sequencing (scChIP-Seq), single-cell Hi-C sequencing (scHI-C-Seq), and single-cell nucleosome occupancy and methylome sequencing (scNOMe-Seq). The single-cell transcriptome can be explored by single-cell RNA sequencing (scRNA-Seq), fluorescence in situ hybridization coupled with flow cytometry (FISH-Flow), and single-cell reverse transcription followed by quantitative PCR (scRT-qPCR). Single-cell technologies to explore the epitranscriptome and translatome have not been developed yet. The study of the proteome at single-cell resolution can be performed by cytometry time-of-flight (CyTOF) and cellular indexing of transcriptomes and epitopes by sequencing (CITE-Seq). Single-cell proteomes and modified proteomes can be analyzed by single-cell proteomics by mass spectrometry (SCoPE-MS). Abbreviation: NA, not applicable.

two main origins: biological and technical. Cellular variability is due to variations in cell-specific expression programs (cell lineage, cell state) and stochastic gene expression. Technical variability is instead due to the incomplete capture of all cellular mRNAs and sequencing depth. Thus, the major challenge of scRNA-Seq is data analysis aimed at minimizing the technical variability, starting from barcode processing, read mapping to a genome or transcriptome reference, raw count matrix generation (gene/UMI counting), preprocessing, normalization, and differential expression analysis (36–38). In a recent study, Vieth et al. (37) evaluated multiple scRNA-Seq pipelines and identified library preparation protocols and normalization as having the greatest effect on the analysis quality. They further recommended analyzing UMI-containing scRNA-Seq data using genome mapping with GENCODE annotation and the splice-aware aligner STAR, preprocessing for gene dropout using SAVER imputation, normalization benefiting from spike-in inclusion and using scran with prior clustering, and differential expression testing using limma-trend (37).

Finally, scRNA-Seq data can be visualized in a two-dimensional plot upon dimensionality reduction, ranging from principal component analysis (PCA) plots for low complexity samples, to t-distributed stochastic neighbor embedding (t-SNE) plots for more complex samples, and more recently to the preferred and more comprehensive uniform manifold approximation and projection (UMAP) plots (38).

2.3. Single-Cell Limitations

Single-cell analyses offer ways to explore cellular heterogeneity per se, as well as cellular heterogeneity in response to a viral infection, providing unprecedented opportunities to identify novel players in the virus-host interplay (6). However, it is important to keep in mind the limitations associated with these technologies: technological restrictions, phenotype association, and possible time-lapse between the single-cell analysis and the phenotype.

Indeed, single-cell technologies are powerful but suffer from multiple technical constraints, such as the manipulation leading to single-cell isolation, the one-shot analysis of a number of limited molecules or parameters, and the technical sensitivity. Exploring the cell-omic content reveals cell heterogeneity but is poorly informative if it is not related to a specific phenotype for the analysis, which needs to be identified and measured as accurately as possible. Finally, it is important to consider the possible temporal gap between the time at which the single-cell analysis is performed and the time at which the associated phenotype is observed, as cell-omic content may change. Indeed, it is difficult to characterize the transcriptomic features of a permissive cell at the time of infection before knowing if the cell is indeed truly permissive and thus infected, for instance.

3. HETEROGENEOUS CELLS PROVIDE HETEROGENEOUS RESPONSES TO HIV

To date, in the field of HIV, single-cell technologies have been mostly used to identify specific gene signatures in the following topics: the cell permissiveness to HIV infection, the latent HIV-infected cell and its reactivation, and the control of HIV infection by immune cells (**Figure 3**). An overview of the main findings is summarized here.

3.1. HIV Permissiveness

Infection outcome is determined by the cell content and results from a balance between cellular players promoting or restricting HIV progression throughout the cell. To successfully replicate, HIV encodes 15 proteins, each of which is able to interact with a plethora of cellular proteins (39, 40). On one hand, HIV hijacks cellular factors, termed HIV dependency factors (HDFs), to favor its own replication (41). On the other hand, the cell possesses innate immune defenses, with HIV restriction factors and HIV inhibitory factors (HIFs), which are however often counteracted by HIV proteins (42–45). The identification of HDFs and HIFs informs on HIV life cycle but also provides novel opportunities for targeted antiviral development. Exploiting the cell heterogeneity toward HIV infection should help researchers gain more insights into HIV replication and the role of novel cellular factors.

$CD4^+$ T cell permissiveness to HIV infection can be observed using diverse approaches. Methodologies based on time-lapse microscopy, FACS, three-dimensional immuno-DNA fluorescence, and other visualization techniques provide many examples of cellular heterogeneity toward HIV infection, informing on many viral and kinetic features, such as time for early and late gene expression, nuclear location of proviruses, and viral RNA transcription, but without characterizing the permissive cell per se (46–56).

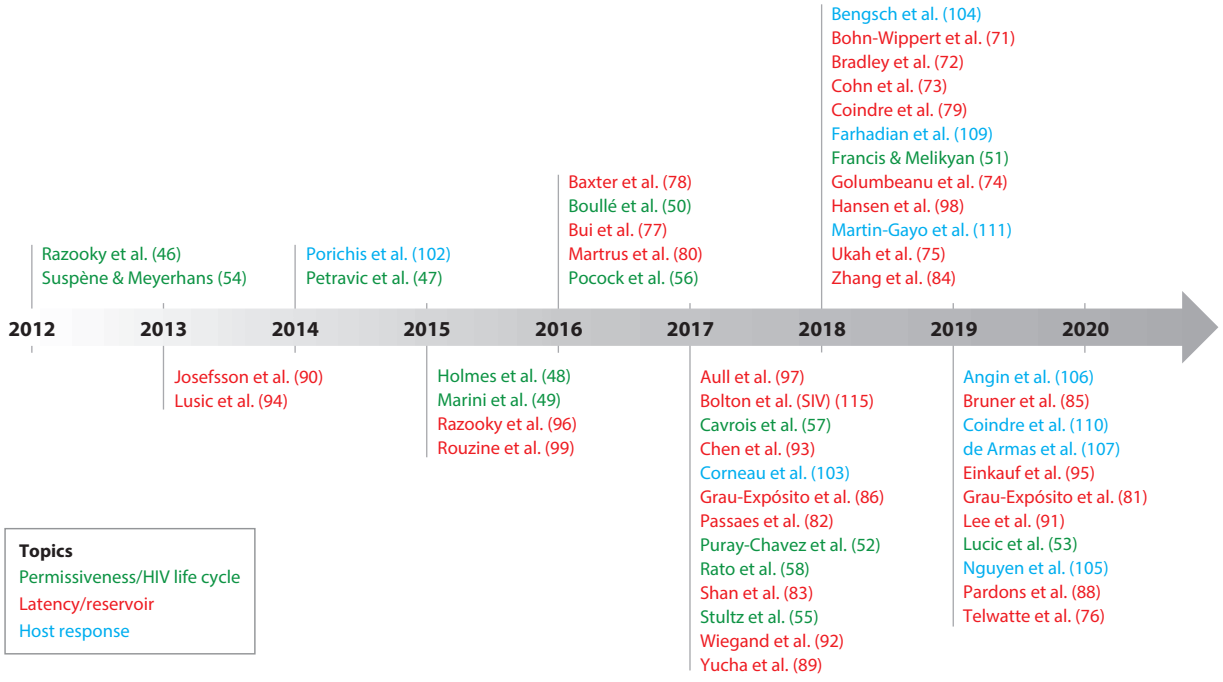


Figure 3

Timeline of human immunodeficiency virus (HIV) single-cell studies. In recent years, the number of studies using single-cell technologies has exponentially increased in the field of HIV, improving the knowledge of HIV life cycle and permissiveness (*green*), HIV latency and reservoir (*red*), and host immune response to HIV (*blue*).

Attempts at characterizing permissiveness were recently achieved by two studies using two different approaches (**Figure 4**). In a first study, Cavrois et al. (57) used mass cytometry or cytometry by time-of-flight (CyTOF) to investigate tonsillar $CD4^+$ T cell permissiveness using 38 parameters, aimed at identifying $CD4^+$ T cell subsets, activation status, and some receptors. They used a beta-lactamase (BlaM)-Vpr HIV virus encoding the murine heat stable antigen (HSA) as late reporter. The incorporation of BlaM-Vpr protein in the virion allows the rapid detection, within a 2-h time window, of viral entry upon virus fusion at the cell membrane, via intracellular cleavage of a fluorescent BlaM substrate, and subsequent sorting by FACS before CyTOF analysis. This analysis showed that HIV virions did not enter naïve $CD4^+$ T cells ($CD4^+CD45RA^+CD45RO^-$), likely due to low CCR5 expression, but entered all memory $CD4^+$ T cells ($CD4^+CD45RA^-CD45RO^+$) efficiently. In particular, virion fusion occurred more efficiently in Th2-like, Th17-like, and T regulatory cells. Cell permissiveness was then assessed by looking at virus-encoding HSA after 4 days of infection as a surrogate of successful viral expression. Although viruses entered all memory $CD4^+$ T cells, Th17-like ($CCR6^+CCR4^+$) and Tfh ($PD1^+CXCR5^+$) cells were more permissive to HIV replication and were further characterized by expression of CD69, CD38, PD1, CD57, and low CD127. In the second study, Rato et al. (58) used full-length scRNA-Seq to profile 166 $CD4^+$ T cells from two donors displaying opposite permissiveness phenotypes, correlating the transcriptome to cell surface protein expression and permissiveness. Their analysis identified highly permissive cells as expressing high levels of CD25, CD298, CD63, and CD317 and that were characterized at the transcriptome level by downregulation of innate immunity components such as genes involved in the type I interferon (IFN) pathway and certain other restriction factors.

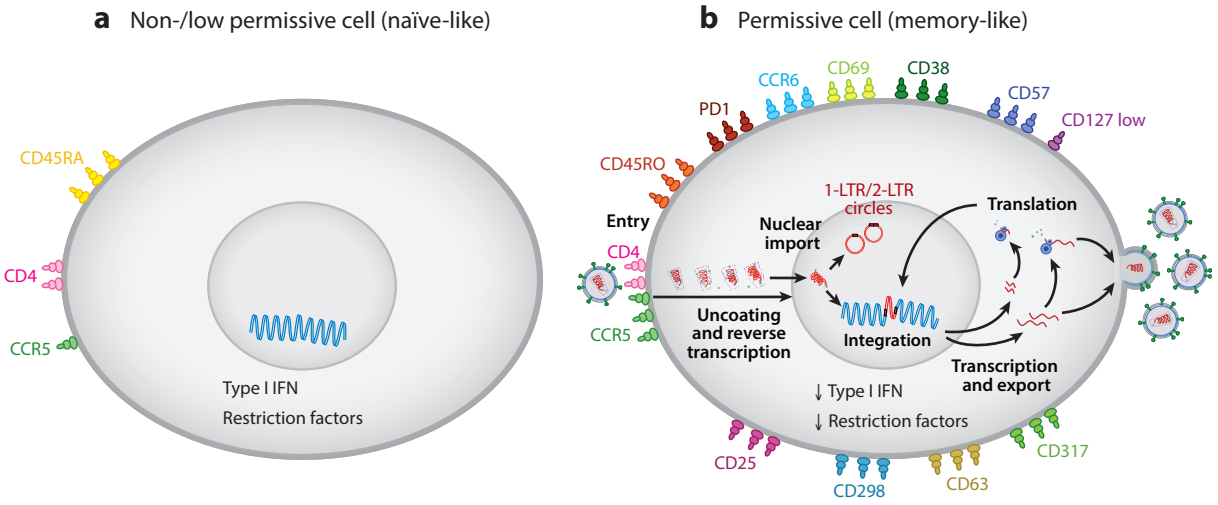


Figure 4

Determinants of human immunodeficiency virus (HIV) permissiveness. (*a*) Naïve CD4$^+$ T cells are mostly nonpermissive, mainly due to low C-C chemokine receptor (CCR) 5 expression. (*b*) In contrast, memory CD4$^+$ T cells are more permissive to HIV infection. However, Th17-like (CCR6$^+$CCR4$^+$) and Tfh (PD1$^+$CXCR5$^+$) cells expressing some activation markers (CD69, CD38), CD57, and low levels of CD127 (the interleukin-7 receptor) were shown to be the most permissive. Using another approach, CD4$^+$ T cells expressing high levels of CD25, CD298, CD63, and CD317 cell surface molecules corresponded to cells with impaired type I interferon (IFN) response and reduced expression of some HIV restriction factors, consistent with a permissive phenotype to HIV infection.

These studies are complementary and point toward an active cellular state and impaired immune response factors as determinants of HIV permissiveness.

3.2. HIV Pathogenicity: Latency Versus Reactivated or Productive Viral Expression

Although combined antiretroviral therapy (ART) allows efficiently stopping HIV disease progression, bringing plasma viremia to undetectable levels, it cannot completely get rid of the virus (59, 60). Indeed, upon ART cessation, the virus rebounds typically within 2–3 weeks, indicating the presence of a persistent viral reservoir (59–63). This reservoir is established very quickly upon virus transmission, within the first 2 to 3 days, and decays very slowly, thereby requiring life-long treatment for HIV$^+$ individuals and representing the major obstacle to an HIV cure (64–66). To date, the viral reservoir is characterized by the persistence of long-lived infected cells that contain an integrated copy of the viral genome and that are not eliminated, either because they reside in anatomical sanctuaries or because they are latent, i.e., with reduced viral protein expression and replication in these cells (67–70). Understanding all the features of this latent reservoir is thus considered to be essential in the development of targeted strategies aiming at its eradication (67–70).

The latent reservoir is very complex and heterogeneous as (*a*) the infected cell subset varies and thus differs in its transcriptomic and proteomic cell content, including naïve CD4$^+$ T cells, memory CD4$^+$ T cells (such as central or memory), or non-T cells (such as macrophages); (*b*) the cell state can vary, from resting to active or cycling status; and (*c*) the integration site of the HIV genome differs from one cell to another. This variability, in turn, affects the reactivation potential of latently infected cells, i.e., the potential for efficient induction of HIV gene product expression.

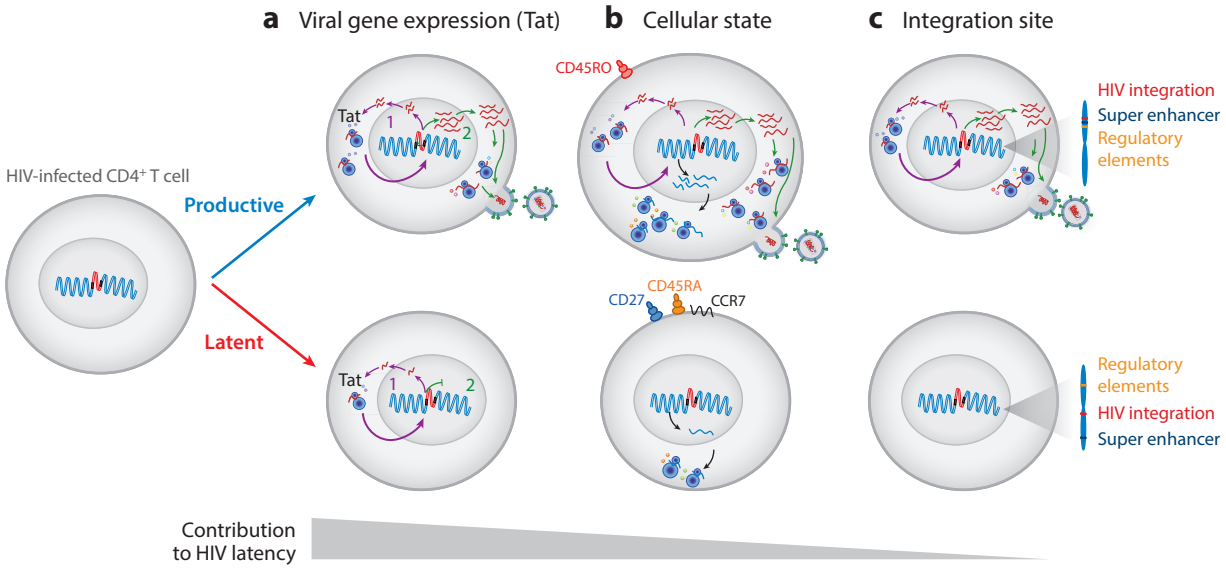


Figure 5

Relevant features of latently infected CD4$^+$ T cells. Human immunodeficiency virus (HIV)-infected CD4$^+$ T cells can be in either a productive or latent state. The cell fate can be influenced by three major contributors: (*a*) the expression level of the early viral Tat protein (*purple arrows*) that either can be insufficient to boost viral transcription (latent cell) or can successfully trigger the late phase of gene expression (*green arrows*) and allow viral particle production, (*b*) the cellular state of the cell can provide different cellular environments that can support and promote viral particle production or not, and (*c*) the provirus genomic context that can affect viral gene expression. Proviral insertions located close to super enhancers and certain other regulatory elements are more likely to be transcriptionally active. Abbreviation: CCR, C-C chemokine receptor.

Single-cell studies are thus instrumental to explore the heterogeneity of HIV latency establishment and reactivation. In particular, three aspects of virus-host interaction can be investigated with single-cell technologies: (*a*) host cell environment and genetic makeup, (*b*) the integration site location and nuclear location, and (*c*) viral gene expression (**Figure 5**).

Single-cell transcriptomic analyses were used to profile latent cells, characterizing cell heterogeneity and the associated transcriptional environment (71–76). Bradley et al. (72) used a primary cell latency model and analyzed a total of 4,206 latent CD4$^+$ T cells from three donors. They compared cellular transcriptomes of GFP^{high} vRNA^{high} cells versus GFP^{neg} vRNA^{low} bona fide latent cells and found 89 upregulated transcripts and 42 downregulated transcripts. Genes upregulated in the GFP^{high} vRNA^{high} productive population were consistent with an activated T cell state (CD25, OX40L, GITR) while the GFP^{neg} vRNA^{low} population was enriched in markers consistent with a naïve (Tn) or central memory (Tcm) phenotype (CCR7, CXCR4, CD62L, CD127).

One strategy to overcome latency consists of reactivating virus expression to purge the viral reservoir. Latency reversal agents (LRAs) can reactivate only a limited fraction of latently infected cells, confirming some degree of heterogeneity in the latent cell population. Golumbeanu et al. (74) explored transcriptional heterogeneity of 224 latently infected cells upon different reactivation conditions using scRNA-Seq in a primary cell latency model. They identified a 134-gene signature characterizing the inducible latent cell, showing enrichment for metabolism. Consistent with this, Bohn-Wippert et al. (71) and Cohn et al. (73) showed that cell size and cellular metabolic activity positively correlated with reactivation potential of latently infected cells, e.g.,

large and metabolically active cells were more prone to reactivation (71, 72, 74). Furthermore, scRNA-Seq analysis of latent or phytohaemagglutinin-reactivated $CD4^+$ T cells isolated from HIV-1^+ blood donors revealed enrichment for gene ontology immune system functions (73).

Single-cell flow-based and single-cell RT-qPCR techniques were extensively used to explore correlation between T cell subsets and latency reactivation potential (77–86). Overall, these studies suggested that cells displaying an effector memory (Tem) or transition memory (Ttm) phenotype were a niche for transcriptional and translational competent reservoirs (81, 87–89). Furthermore, LRA-mediated HIV reactivation was shown to be heterogeneous, acting by either increasing transcription from active cells or increasing the number of transcriptionally active cells (89). An in-depth investigation of multiple LRAs and their ability to reactivate latently infected cells isolated from ART-treated HIV^+ individuals showed that only 2.6% of HIV-1 latent proviruses were completely reactivated upon LRA stimulation (81). Moreover, they showed that different drugs had different effects according to the T cell subset, i.e., Panobinostat successfully reactivated HIV in Tcm cells only while Romidepsin affected all memory subsets.

Nevertheless, all these studies pointed out a similar observation: Only a fraction of cells were efficiently reactivated, regardless of the T cell subset or cellular environment, indicating that other factors, such as proviral DNA integrity, integration site, or stochasticity, likely also contribute to latency establishment.

Several studies took advantage of full-length single-genome sequences to explore the implication of provirus DNA and its integration site location in latency (90–92). These studies described that the majority of latently infected cells contain only one provirus and that HIV proviruses identified in different cell subsets and in different anatomical sites were genetically similar, potentially suggesting clonality from a common progenitor ancestor. Furthermore, Wiegand et al. (92) investigated the proportion of transcriptionally active cells in 3 ART-treated HIV^+ individuals. They performed cell-associated HIV RNA and DNA single-genome sequencing (CARD-SGS) and found out that 7% of proviruses expressed HIV RNA, ranging from 1 to 62 HIV molecules/cell (median of 1).

Integration site location, considered at the level of both chromosomal context and nuclear topology, can contribute to HIV latency (93–95). Chen et al. (93) used a single virus tracking technique (Barcoded HIV ensembles, or B-HIVE) to show that integration sites close to enhancers resulted in higher provirus expression levels. Although comparison of HIV integration site location between latently and productively infected cells did not reveal any difference, distance from enhancers was on average two times further for latent proviruses, indicating an effect of integration site location on provirus expression (93). These findings were confirmed by Einkauf et al. (95), who used Matched Integration Site and Proviral Sequencing (MIP-Seq) to investigate integration site location and proviral sequence at sc level in 3 ART-treated patients. They observed that intact proviruses were enriched in non-genic regions and found in the opposite orientation with respect to host genes.

HIV genome position within the nucleus can also affect gene expression. Indeed, HIV proviruses colocalized within nuclear bodies in latently infected cells, and such colocalization was lost upon LRA reactivation (94).

Viral transcription, hence viral reactivation, is highly dependent on the HIV transactivator of transcription, Tat. Indeed, accumulation of Tat boosts viral transcription and favors viral production.

Studies of the efficiency of viral expression benefited from fluorescence reporter-based single-cell techniques, identifying two major determinants of latent or productive cell fate: (*a*) initial determination of basal level of Tat expression due to stochasticity and Tat accumulation and

(*b*) direct Tat-mediated effect on viral transcription (92, 96–98). In case of low Tat amounts, viral gene expression depends mainly on the cellular gene expression context. In contrast, high Tat levels triggered a positive feedback loop where virus expression is maintained even in case of cellular reversion to a resting stage, suggesting that the Tat feedback loop dictated HIV expression, overcoming the cellular state influence (96, 98).

Altogether, Tat expression, host cell environment, and integration site location can all affect viral gene expression to various degrees.

Latency has often been perceived as an evolutionary mistake; however, Rouzine et al. (99) used advanced modeling strategies to show that latency gives an evolutionary advantage to lentiviruses by facilitating the spread in target-cell-poor environment (i.e., mucosa) and increasing survival of the virus.

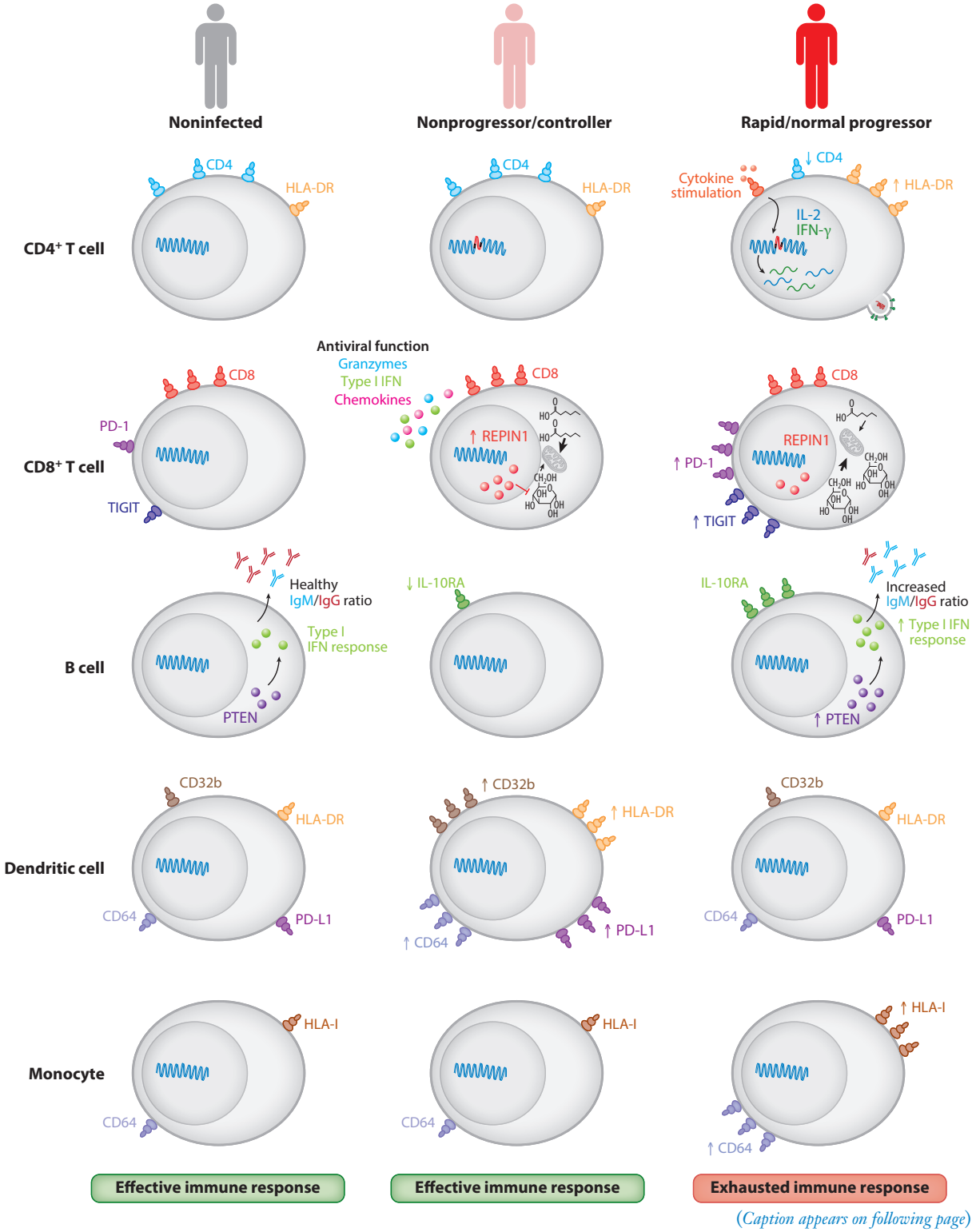
3.3. Host Response to HIV: Immune Control from Immune Cells

As mentioned in **Figure 1*a***, individuals do not respond equally toward HIV infection. This can be due to differential $CD4^+$ T cell susceptibility to HIV infection but also to differential immune control capacity potentially involving a variety of immune cells, including $CD8^+$ T cells, dendritic cells (DCs), $CD4^+$ T helper cells, and B cells (100, 101). Efficient immune response against viral infection can be characterized by antiviral signaling molecules (IFNs, cytokines, or chemokines), antibody production, and cytotoxic response. A minority of HIV^+ individuals, named elite controllers (ECs), are able to control HIV infection and preserve their immune functions. However, HIV overtakes the immune response in most HIV^+ individuals (normal/rapid progressors), leading to exhaustion and depletion of the immune system (102–111) (**Figure 6**).

Single-cell analysis of 96 subset-specific genes was performed on 1,440 individual $CD8^+$ Tcm cells isolated from controllers or ART-treated progressors and revealed opposite patterns of gene expression (106). $CD8^+$ Tcm cells isolated from HIV controllers displayed overexpression of effector function genes (*GZMB*, *GZK*, *CCL3*, *CCL3L1*, *XCL1*), survival genes (*CD69*, *KLRD1*), CTL-induced apoptosis genes (*FASLG*, *TNF*, *TRAIL*), and *IFNB*. Furthermore, these cells were able to use energy sources (i.e., fatty acids) other than glucose. In contrast, $CD8^+$ Tcm cells isolated from ART-treated progressors displayed increased activation, increased exhaustion (*LAG3*) increased glycolysis and dependence on glucose as the sole energy source, and increased expression of IFN-stimulated genes (*MX1*, *OAS1*). Of note, glucose dependency can be inhibited upon IL-15 exposure and downregulation of the REPIN-1 transcription factor, responsible for downregulation of glucose transport.

Upon analysis of HIV-infected peripheral blood mononuclear cells from three ECs, a subset of myeloid dendritic cells (mDCs) displaying an antiviral state was identified and further characterized by scRNA-Seq (111). These antiviral mDCs were characterized by high expression of PD-L1 and CD64, by the ability to stimulate IFNγ response, and by degranulation markers (LAMP1/CD107a, TNFα) from autologous $CD8^+$ T cells. This antiviral mDC profile can be stimulated by TLR3 and poly:IC. Moreover, Coindre et al. (110) used CyTOF to characterize monocytes and DCs from blood myeloid cells. They identified that specific classical dendritic cell (cDC) clusters, expressing high levels of $CD1c^+$, CD32b, and HLA-DR, can be associated with elite control.

Chronic infection leads to immune cell exhaustion, resulting in functional defects and expression of inhibitory receptors, that can be investigated by CyTOF analysis (104). HIV-induced $CD8^+$ T cell exhaustion is characterized by the expression of inhibitory receptors, such as PD-1 and TIGIT. These cell surface receptors are increased in $CD27^+/CD45RA^-$ effector memory



(*Caption appears on following page*)

Figure 6 (*Figure appears on preceding page*)

Host immune control of human immunodeficiency virus (HIV). Comparative single-cell analyses on immune cells derived from noninfected individuals (*gray*), controller/nonprogressor HIV$^+$ individuals (*light red*), and rapid/normal progressors (*red*). Single-cell analyses were performed on only two sample subsets at a time, either noninfected individuals versus normal/rapid progressors or controllers/nonprogressors versus normal/rapid progressors. Thus, the absence of a specific marker in a sample subset panel without indication reflects a lack of available data or information in the literature. Immune cells isolated from noninfected individuals or HIV controllers/nonprogressors displayed effective immune response, while cells isolated from rapid or normal progressors showed an exhausted phenotype. Abbreviations: HLA, human leukocyte antigen; IFN, interferon; IgM/IgG, immunoglobulin M to immunoglobulin G.

CD8$^+$ T cells. In contrast, CD8$^+$ T cell function is preserved in ECs and is associated with inhibitory receptor downregulation, cytolytic molecule downregulation, and cytokine upregulation, which are typical of a functional memory phenotype with a poor but potent cytotoxic activity (105).

Most HIV pathogenesis studies focus on T cells, as they are the main targets of HIV. However, despite not being infected by HIV, B cells' function can be altered in the presence of HIV-infected cells. Indeed, upon H1N1 vaccination, single-cell reverse transcription PCR highlighted an immunoglobulin G to immunoglobulin M production switch in HIV-infected individuals but not in healthy donors (107). This impairment in antibody response is likely the result of the enhanced IFN-I response, leading to B cell exhaustion. Thus, it is tempting to think that restoring B cell function could partially contribute to antibody-mediated control of HIV infection.

4. CONCLUDING REMARKS AND PERSPECTIVES

Single-cell analyses provide the unprecedented opportunity to study one cell at a time, one layer at a time, and one phenotype at a time. Further integrating additional layers using single-cell multi-omics technologies should allow advancing one step forward to a more comprehensive picture of the HIV-infected cell and of host immune control. The analysis of cell heterogeneity toward HIV infection has the potential to identify novel cellular players affecting HIV replication, either promoting or inhibiting it. In addition, analysis of host immune control should help uncover the molecular determinants leading to successful control. Thus, single-cell analyses should provide further cues on HIV replication as well as pave the way for novel targeted therapeutic interventions.

Moreover, these HIV studies provide useful and valuable models to be extended to other viruses. Such single-cell studies are being pursued with a growing number of viruses for varied purposes, especially in the realm of single-cell RNA sequencing (e.g., 112–115). The many examples outlined here for HIV show the rich potential for developing and fruitfully integrating a broad array of diverse single-cell studies in order to fully characterize the replication and host interactions of essentially all viruses and exploit these results to improve virus control.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Telenti A, Goldstein DB. 2006. Genomics meets HIV-1. *Nat. Rev. Microbiol.* 4:865–73
2. Fellay J, Shianna KV, Ge D, Colombo S, Ledergerber B, et al. 2007. A whole-genome association study of major determinants for host control of HIV-1. *Science* 317:944–47
3. McLaren PJ, Carrington M. 2015. The impact of host genetic variation on infection with HIV-1. *Nat. Immunol.* 16:577–83
4. Bartha I, McLaren PJ, Ciuffi A, Fellay J, Telenti A. 2014. GuavaH: a compendium of host genomic data in HIV biology and disease. *Retrovirology* 11:6
5. Bartha I, McLaren PJ, Brumme C, Harrigan R, Telenti A, Fellay J. 2017. Estimating the respective contributions of human and viral genetic variation to HIV control. *PLOS Comput. Biol.* 13:e1005339
6. Cristinelli S, Ciuffi A. 2018. The use of single-cell RNA-Seq to understand virus-host interactions. *Curr. Opin. Virol.* 29:39–50
7. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, et al. 2001. The sequence of the human genome. *Science* 291:1304–51
8. Tang F, Barbacioru C, Wang Y, Nordman E, Lee C, et al. 2009. mRNA-Seq whole-transcriptome analysis of a single cell. *Nat. Methods* 6:377–82
9. Gross A, Schoendube J, Zimmermann S, Steeb M, Zengerle R, Koltay P. 2015. Technologies for single-cell isolation. *Int. J. Mol. Sci.* 16:16897–919
10. Mincarelli L, Lister A, Lipscombe J, Macaulay IC. 2018. Defining cell identity with single-cell omics. *Proteomics* 18:e1700312
11. Svensson V, Vento-Tormo R, Teichmann SA. 2018. Exponential scaling of single-cell RNA-seq in the past decade. *Nat. Protoc.* 13:599–604
12. Hu P, Zhang W, Xin H, Deng G. 2016. Single cell isolation and analysis. *Front. Cell Dev. Biol.* 4:116
13. Tanay A, Regev A. 2017. Scaling single-cell genomics from phenomenology to mechanism. *Nature* 541:331–38
14. Gawad C, Koh W, Quake SR. 2016. Single-cell genome sequencing: current state of the science. *Nat. Rev. Genet.* 17:175–88
15. Vitak SA, Torkenczy KA, Rosenkrantz JL, Fields AJ, Christiansen L, et al. 2017. Sequencing thousands of single-cell genomes with combinatorial indexing. *Nat. Methods* 14:302–8
16. Wen L, Tang F. 2018. Single cell epigenome sequencing technologies. *Mol. Aspects Med.* 59:62–69
17. Mulqueen RM, Pokholok D, Norberg SJ, Torkenczy KA, Fields AJ, et al. 2018. Highly scalable generation of DNA methylation profiles in single cells. *Nat. Biotechnol.* 36:428–31
18. Karemaker ID, Vermeulen M. 2018. Single-cell DNA methylation profiling: technologies and biological applications. *Trends Biotechnol.* 36:952–65
19. Kanter I, Kalisky T. 2015. Single cell transcriptomics: methods and applications. *Front. Oncol.* 5:53
20. Ziegenhain C, Vieth B, Parekh S, Reinius B, Guillaumet-Adkins A, et al. 2017. Comparative analysis of single-cell RNA sequencing methods. *Mol. Cell* 65:631–43
21. Kulkarni A, Anderson AG, Merullo DP, Konopka G. 2019. Beyond bulk: a review of single cell transcriptomics methodologies and applications. *Curr. Opin. Biotechnol.* 58:129–36
22. Cao J, Packer JS, Ramani V, Cusanovich DA, Huynh C, et al. 2017. Comprehensive single-cell transcriptional profiling of a multicellular organism. *Science* 357:661–67
23. Rosenberg AB, Roco CM, Muscat RA, Kuchina A, Sample P, et al. 2018. Single-cell profiling of the developing mouse brain and spinal cord with split-pool barcoding. *Science* 360:176–82
24. Chen KH, Boettiger AN, Moffitt JR, Wang S, Zhuang X. 2015. Spatially resolved, highly multiplexed RNA profiling in single cells. *Science* 348:aaa6090
25. Kolodziejczyk AA, Lonnberg T. 2018. Global and targeted approaches to single-cell transcriptome characterization. *Brief Funct. Genom.* 17:209–19
26. Wu M, Singh AK. 2012. Single-cell protein analysis. *Curr. Opin. Biotechnol.* 23:83–88
27. Su Y, Shi Q, Wei W. 2017. Single cell proteomics in biomedicine: high-dimensional data acquisition, visualization, and analysis. *Proteomics* 17:1600267
28. Levy E, Slavov N. 2018. Single cell protein analysis for systems biology. *Essays Biochem.* 62:595–605

29. Macaulay IC, Ponting CP, Voet T. 2017. Single-cell multiomics: multiple measurements from single cells. *Trends Genet*. 33:155–68
30. Stuart T, Satija R. 2019. Integrative single-cell analysis. *Nat. Rev. Genet.* 20:257–72
31. Chen W, Li S, Kulkarni AS, Huang L, Cao J, et al. 2020. Single cell omics: from assay design to biomedical application. *Biotechnol. J.* 15:e1900262
32. Angermueller C, Clark SJ, Lee HJ, Macaulay IC, Teng MJ, et al. 2016. Parallel single-cell sequencing links transcriptional and epigenetic heterogeneity. *Nat. Methods* 13:229–32
33. Hu Y, Huang K, An Q, Du G, Hu G, et al. 2016. Simultaneous profiling of transcriptome and DNA methylome from a single cell. *Genome Biol*. 17:88
34. Bock C, Farlik M, Sheffield NC. 2016. Multi-omics of single cells: strategies and applications. *Trends Biotechnol*. 34:605–8
35. Hu Y, An Q, Sheu K, Trejo B, Fan S, Guo Y. 2018. Single cell multi-omics technology: methodology and application. *Front. Cell Dev. Biol.* 6:28
36. Ziegenhain C, Vieth B, Parekh S, Hellmann I, Enard W. 2018. Quantitative single-cell transcriptomics. *Brief Funct. Genom.* 17:220–32
37. Vieth B, Parekh S, Ziegenhain C, Enard W, Hellmann I. 2019. A systematic evaluation of single cell RNA-seq analysis pipelines. *Nat. Commun.* 10:4667
38. Choi YH, Kim JK. 2019. Dissecting cellular heterogeneity using single-cell RNA sequencing. *Mol. Cells* 42:189–99
39. van Dijk D, Ertaylan G, Boucher CA, Sloot PM. 2010. Identifying potential survival strategies of HIV-1 through virus-host protein interaction networks. *BMC Syst. Biol.* 4:96
40. Ptak RG, Fu W, Sanders-Beer BE, Dickerson JE, Pinney JW, et al. 2008. Cataloguing the HIV type 1 human protein interaction network. *AIDS Res. Hum. Retroviruses* 24:1497–502
41. Bushman FD, Malani N, Fernandes J, D'Orso I, Cagney G, et al. 2009. Host cell factors in HIV replication: meta-analysis of genome-wide studies. *PLOS Pathog*. 5:e1000437
42. Yin X, Langer S, Zhang Z, Herbert KM, Yoh S, et al. 2020. Sensor sensibility—HIV-1 and the innate immune response. *Cells* 9:254
43. Schoggins JW. 2019. Interferon-stimulated genes: What do they all do? *Annu. Rev. Virol.* 6:567–84
44. Bergantz L, Subra F, Deprez E, Delelis O, Richetta C. 2019. Interplay between intrinsic and innate immunity during HIV infection. *Cells* 8:922
45. Sauter D, Kirchhoff F. 2019. Key viral adaptations preceding the AIDS pandemic. *Cell Host Microbe* 25:27–38
46. Razooky BS, Gutierrez E, Terry VH, Spina CA, Groisman A, Weinberger LS. 2012. Microwell devices with finger-like channels for long-term imaging of HIV-1 expression kinetics in primary human lymphocytes. *Lab. Chip* 12:4305–12
47. Petravic J, Ellenberg P, Chan ML, Paukovics G, Smyth RP, et al. 2014. Intracellular dynamics of HIV infection. *J. Virol.* 88:1113–24
48. Holmes M, Zhang F, Bieniasz PD. 2015. Single-cell and single-cycle analysis of HIV-1 replication. *PLOS Pathog*. 11:e1004961
49. Marini B, Kertesz-Farkas A, Ali H, Lucic B, Lisek K, et al. 2015. Nuclear architecture dictates HIV-1 integration site selection. *Nature* 521:227–31
50. Boullé M, Muller TG, Dahling S, Ganga Y, Jackson L, et al. 2016. HIV cell-to-cell spread results in earlier onset of viral gene expression by multiple infections per cell. *PLOS Pathog*. 12:e1005964
51. Francis AC, Melikyan GB. 2018. Single HIV-1 imaging reveals progression of infection through CA-dependent steps of docking at the nuclear pore, uncoating, and nuclear transport. *Cell Host Microbe* 23:536–48
52. Puray-Chavez M, Tedbury PR, Huber AD, Ukah OB, Yapo V, et al. 2017. Multiplex single-cell visualization of nucleic acids and protein during HIV infection. *Nat. Commun.* 8:1882
53. Lucic B, Chen HC, Kuzman M, Zorita E, Wegner J, et al. 2019. Spatially clustered loci with multiple enhancers are frequent targets of HIV-1 integration. *Nat. Commun.* 10:4059
54. Suspène R, Meyerhans A. 2012. Quantification of unintegrated HIV-1 DNA at the single cell level in vivo. *PLOS ONE* 7(5):e36246

55. Stultz RD, Cenker JJ, McDonald D. 2017. Imaging HIV-1 genomic DNA from entry through productive infection. *J. Virol.* 91(9):e00034-17
56. Pocock GM, Zimdars LL, Yuan M, Eliceiri KW, Ahlquist P, Sherer NM. 2016. Diverse activities of viral *cis*-acting RNA regulatory elements revealed using multicolor, long-term, single-cell imaging. *Mol. Biol. Cell* 28(3):476–86
57. Cavrois M, Banerjee T, Mukherjee G, Raman N, Hussien R, et al. 2017. Mass cytometric analysis of HIV entry, replication, and remodeling in tissue $CD4^+$ T cells. *Cell Rep*. 20:984–98
58. Rato S, Rausell A, Munoz M, Telenti A, Ciuffi A. 2017. Single-cell analysis identifies cellular markers of the HIV permissive cell. *PLOS Pathog*. 13:e1006678
59. Durand CM, Blankson JN, Siliciano RF. 2012. Developing strategies for HIV-1 eradication. *Trends Immunol*. 33:554–62
60. Spivak AM, Planelles V. 2016. HIV-1 eradication: early trials (and tribulations). *Trends Mol. Med.* 22:10–27
61. Rothenberger MK, Keele BF, Wietgrefe SW, Fletcher CV, Beilman GJ, et al. 2015. Large number of rebounding/founder HIV variants emerge from multifocal infection in lymphatic tissues after treatment interruption. *PNAS* 112:E1126–34
62. Colby DJ, Trautmann L, Pinyakorn S, Leyre L, Pagliuzza A, et al. 2018. Rapid HIV RNA rebound after antiretroviral treatment interruption in persons durably suppressed in Fiebig I acute HIV infection. *Nat. Med.* 24:923–26
63. Van Lint C, Bouchat S, Marcello A. 2013. HIV-1 transcription and latency: an update. *Retrovirology* 10:67
64. Whitney JB, Hill AL, Sanisetty S, Penaloza-MacMaster P, Liu J, et al. 2014. Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys. *Nature* 512:74–77
65. Whitney JB, Lim SY, Osuna CE, Kublin JL, Chen E, et al. 2018. Prevention of SIVmac251 reservoir seeding in rhesus monkeys by early antiretroviral therapy. *Nat. Commun.* 9:5429
66. Bachmann N, von Siebenthal C, Vongrad V, Turk T, Neumann K, et al. 2019. Determinants of HIV-1 reservoir size and long-term dynamics during suppressive ART. *Nat. Commun.* 10:3193
67. Falcinelli SD, Ceriani C, Margolis DM, Archin NM. 2019. New frontiers in measuring and characterizing the HIV reservoir. *Front. Microbiol.* 10:2878
68. Pitman MC, Lau JSY, McMahon JH, Lewin SR. 2018. Barriers and strategies to achieve a cure for HIV. *Lancet HIV* 5:e317–28
69. Sengupta S, Siliciano RF. 2018. Targeting the latent reservoir for HIV-1. *Immunity* 48:872–95
70. Ait-Ammar A, Kula A, Darcis G, Verdikt R, De Wit S, et al. 2019. Current status of latency reversing agents facing the heterogeneity of HIV-1 cellular and tissue reservoirs. *Front. Microbiol.* 10:3060
71. Bohn-Wippert K, Tevonian EN, Lu Y, Huang MY, Megaridis MR, Dar RD. 2018. Cell size-based decision-making of a viral gene circuit. *Cell Rep*. 25:3844–57
72. Bradley T, Ferrari G, Haynes BF, Margolis DM, Browne EP. 2018. Single-cell analysis of quiescent HIV infection reveals host transcriptional profiles that regulate proviral latency. *Cell Rep*. 25:107–17
73. Cohn LB, da Silva IT, Valieris R, Huang AS, Lorenzi JCC, et al. 2018. Clonal $CD4^+$ T cells in the HIV-1 latent reservoir display a distinct gene profile upon reactivation. *Nat. Med.* 24:604–9
74. Golumbeanu M, Cristinelli S, Rato S, Munoz M, Cavassini M, et al. 2018. Single-cell RNA-Seq reveals transcriptional heterogeneity in latent and reactivated HIV-infected cells. *Cell Rep*. 23:942–50
75. Ukah OB, Puray-Chavez M, Tedbury PR, Herschhorn A, Sodroski JG, Sarafianos SG. 2018. Visualization of HIV-1 RNA transcription from integrated HIV-1 DNA in reactivated latently infected cells. *Viruses* 10:534
76. Telwatte S, Morón-López S, Aran D, Kim P, Hsieh C, et al. 2019. Heterogeneity in HIV and cellular transcription profiles in cell line models of latent and productive infection: implications for HIV latency. *Retrovirology* 16(1):32
77. Bui JK, Mellors JW, Cillo AR. 2016. HIV-1 virion production from single inducible proviruses following T-cell activation ex vivo. *J. Virol.* 90(3):1673–76
78. Baxter AE, Niessl J, Fromentin R, Richard J, Porichis F, et al. 2016. Single-cell characterization of viral translation-competent reservoirs in HIV-infected individuals. *Cell Host Microbe* 20(3):368–80

79. Coindre S, Tchitchek N, Alaoui L, Vaslin B, Bourgeois C, et al. 2018. Mass cytometry analysis reveals the landscape and dynamics of CD32a$^+$ CD4$^+$ T cells from early HIV infection to effective cART. *Front. Immunol.* 9:1217
80. Martrus G, Niehrs A, Cornelis R, Rechtien A, García-Beltran W, et al. 2016. Kinetics of HIV-1 latency reversal quantified on the single-cell level using a novel flow-based technique. *J. Virol.* 90(20):9018–28
81. Grau-Expósito J, Luque-Ballesteros L, Navarro J, Curran A, Burgos J, et al. 2019. Latency reversal agents affect differently the latent reservoir present in distinct CD4$^+$ T subpopulations. *PLOS Pathog.* 15:e1007991
82. Passaes CP, Bruel T, Decalf J, David A, Angin M, et al. 2017. Ultrasensitive HIV-1 p24 assay detects single infected cells and differences in reservoir induction by latency reversal agents. *J. Virol.* 91(6):e02296-16
83. Shan L, Deng K, Gao H, Xing S, Capoferri AA, et al. 2017. Transcriptional reprogramming during effector-to-memory transition renders CD4$^+$ T cells permissive for latent HIV-1 infection. *Immunity* 47(4):766–75
84. Zhang W, Svensson Akusjärvi S, Sönnerborg A, Neogi U. 2018. Characterization of inducible transcription and translation-competent HIV-1 using the RNAscope ISH technology at a single-cell resolution. *Front. Microbiol.* 9:2358
85. Bruner KM, Wang Z, Simonetti FR, Bender AM, Kwon KJ, et al. 2019. A quantitative approach for measuring the reservoir of latent HIV-1 proviruses. *Nature* 566(7742):120–25
86. Grau-Expósito J, Serra-Peinado C, Miguel L, Navarro J, Curran A, et al. 2017. A novel single-cell FISH-flow assay identifies effector memory CD4$^+$ T cells as a major niche for HIV-1 transcription in HIV-infected patients. *mBio* 8(4):e00876-17
87. Bushman FD, Craigie R. 1990. Sequence requirements for integration of Moloney murine leukemia virus DNA in vitro. *J. Virol.* 64:5645–48
88. Pardons M, Baxter AE, Massanella M, Pagliuzza A, Fromentin R, et al. 2019. Single-cell characterization and quantification of translation-competent viral reservoirs in treated and untreated HIV infection. *PLOS Pathog.* 15:e1007619
89. Yucha RW, Hobbs KS, Hanhauser E, Hogan LE, Nieves W, et al. 2017. High-throughput characterization of HIV-1 reservoir reactivation using a single-cell-in-droplet PCR assay. *EBioMedicine* 20:217–29
90. Josefsson L, Palmer S, Faria NR, Lemey P, Casazza J, et al. 2013. Single cell analysis of lymph node tissue from HIV-1 infected patients reveals that the majority of CD4$^+$ T-cells contain one HIV-1 DNA molecule. *PLOS Pathog.* 9(6):e1003432
91. Lee GQ, Reddy K, Einkauf KB, Gounder K, Chevalier JM, et al. 2019. HIV-1 DNA sequence diversity and evolution during acute subtype C infection. *Nat. Commun.* 10(1):2737
92. Wiegand A, Spindler J, Hong FF, Shao W, Cyktor JC, et al. 2017. Single-cell analysis of HIV-1 transcriptional activity reveals expression of proviruses in expanded clones during ART. *PNAS* 114:E3659–68
93. Chen HC, Martinez JP, Zorita E, Meyerhans A, Filion GJ. 2017. Position effects influence HIV latency reversal. *Nat. Struct. Mol. Biol.* 24:47–54
94. Lusic M, Marini B, Ali H, Lucic B, Luzzati R, Giacca M. 2013. Proximity to PML nuclear bodies regulates HIV-1 latency in CD4$^+$ T cells. *Cell Host Microbe* 13:665–77
95. Einkauf KB, Lee GQ, Gao C, Sharaf R, Sun X, et al. 2019. Intact HIV-1 proviruses accumulate at distinct chromosomal positions during prolonged antiretroviral therapy. *J. Clin. Invest.* 129(3):988–98
96. Razooky BS, Pai A, Aull K, Rouzine IM, Weinberger LS. 2015. A hardwired HIV latency program. *Cell* 160:990–1001
97. Aull KH, Tanner EJ, Thomson M, Weinberger LS. 2017. Transient thresholding: a mechanism enabling noncooperative transcriptional circuitry to form a switch. *Biophys. J.* 112:2428–38
98. Hansen MMK, Wen WY, Ingerman E, Razooky BS, Thompson CE, et al. 2018. A post-transcriptional feedback mechanism for noise suppression and fate stabilization. *Cell* 173:1609–21
99. Rouzine IM, Weinberger AD, Weinberger LS. 2015. An evolutionary role for HIV latency in enhancing viral transmission. *Cell* 160(5):1002–12
100. Walker BD, Yu XG. 2013. Unravelling the mechanisms of durable control of HIV-1. *Nat. Rev. Immunol.* 13:487–98

101. McBrien JB, Kumar NA, Silvestri G. 2018. Mechanisms of $CD8^+$ T cell-mediated suppression of HIV/SIV replication. *Eur. J. Immunol.* 48:898–914
102. Porichis F, Hart MG, Griesbeck M, Everett HL, Hassan M, et al. 2014. High-throughput detection of miRNAs and gene-specific mRNA at the single-cell level by flow cytometry. *Nat. Commun.* 5:5641
103. Corneau A, Cosma A, Even S, Katlama C, Le Grand R, et al. 2017. Comprehensive mass cytometry analysis of cell cycle, activation, and coinhibitory receptors expression in CD4 T cells from healthy and HIV-infected individuals. *Cytometry B Clin. Cytom.* 92:21–32
104. Bengsch B, Ohtani T, Khan O, Setty M, Manne S, et al. 2018. Epigenomic-guided mass cytometry profiling reveals disease-specific features of exhausted CD8 T cells. *Immunity* 48:1029–45
105. Nguyen S, Deleage C, Darko S, Ransier A, Truong DP, et al. 2019. Elite control of HIV is associated with distinct functional and transcriptional signatures in lymphoid tissue $CD8^+$ T cells. *Sci. Transl. Med.* 11(523):eaax4077
106. Angin M, Volant S, Passaes C, Lecuroux C, Monceaux V, et al. 2019. Metabolic plasticity of HIV-specific $CD8^+$ T cells is associated with enhanced antiviral potential and natural control of HIV-1 infection. *Nat. Metab.* 1:704–16
107. de Armas LR, Pallikkuth S, Pan L, Rinaldi S, Cotugno N, et al. 2019. Single cell profiling reveals PTEN overexpression in influenza-specific B cells in aging HIV-infected individuals on anti-retroviral therapy. *Sci. Rep.* 9:2482
108. Han Q, Bradley T, Williams WB, Cain DW, Montefiori DC, et al. 2020. Neonatal rhesus macaques have distinct immune cell transcriptional profiles following HIV envelope immunization. *Cell Rep.* 30:1553–69
109. Farhadian SF, Mehta SS, Zografou C, Robertson K, Price RW, et al. 2018. Single-cell RNA sequencing reveals microglia-like cells in cerebrospinal fluid during virologically suppressed HIV. *JCI Insight* 3:e121718
110. Coindre S, Tchitchek N, Alaoui L, Vaslin B, Bourgeois C, et al. 2019. Mass cytometry analysis reveals complex cell-state modifications of blood myeloid cells during HIV infection. *Front. Immunol.* 10:2677
111. Martin-Gayo E, Cole MB, Kolb KE, Ouyang Z, Cronin J, et al. 2018. A reproducibility-based computational framework identifies an inducible, enhanced antiviral state in dendritic cells from HIV-1 elite controllers. *Genome Biol.* 19:10
112. Zanini F, Robinson ML, Croote D, Sahoo MK, Sanz AM, et al. 2018. Virus-inclusive single-cell RNA sequencing reveals the molecular signature of progression to severe dengue. *PNAS* 115(52):E12363–69
113. Wyler E, Franke V, Menegatti J, Kocks C, Boltengagen A, et al. 2019. Single-cell RNA-sequencing of herpes simplex virus 1-infected cells connects NRF2 activation to an antiviral program. *Nat. Commun.* 10:4878
114. Zhang J, Liu J, Yuan Y, Huang F, Ma R, et al. 2020. Two waves of pro-inflammatory factors are released during the influenza A virus (IAV)-driven pulmonary immunopathogenesis. *PLOS Pathog.* 16(2):e1008334
115. Bolton DL, McGinnis K, Finak G, Chattopadhyay P, Gottardo R, et al. 2017. Combined single-cell quantitation of host and SIV genes and proteins ex vivo reveals host-pathogen interactions in individual cells. *PLOS Pathog.* 13(6):e1006445

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