Immunology

Silence, escape and survival for HIV to persist

Nicolas Chomont

Sophisticated experimental approaches reveal cellular and immune-system mechanisms that enable rare HIV-infected cells to persist for decades in people who are taking antiretroviral drugs. **See p.309 & p.318**

More than 25 years after the life-changing discovery that a combination of antiviral drugs can efficiently suppress the replication of HIV, there is still no cure for the infection. The virus hides in immune cells that contain a copy of the HIV genome integrated into their DNA; such rare types of cell form a population that remains present for a lifetime[1–4]. Writing in *Nature*, Clark *et al.*[5] (page 318) and Sun *et al.*[6] (page 309) unravel the mechanisms by which these HIV-infected cells, known as reservoir cells, can persist.

The number of reservoir cells in an infected person remains relatively stable over time[7,8], and it is unlikely that current antiretroviral therapies (ARTs) alone will be able to eliminate HIV. As such, antiretroviral drugs must be taken for life to prevent resurgence of the virus from these long-lived reservoirs. HIV is found mainly in immune cells called memory CD4$^+$ T cells. Immunological memory can persist for decades owing to the intrinsic capacity of memory CD4$^+$ T cells for survival and proliferation[9,10]. However, whether HIV-infected cells use the same mechanisms to persist as do other long-lived memory T cells, or have different properties, has been unclear.

Clark and colleagues used a microfluidic technology named FIND-seq (described in another paper[11] published simultaneously in *Nature* by Clark *et al.*) to isolate and study reservoir cells. This method allowed the authors to analyse the gene-expression profile (the transcriptome) of cells from three people with HIV who had been receiving ART for years. By embedding single cells into agarose droplets and using a technique to amplify a small section of the HIV genome, if present, Clark and colleagues physically separated cells containing HIV from their uninfected counterparts, and then analysed the cellular RNA of both populations. Two groups of genes, accounting for only 0.81% of the measured transcriptome, distinguished HIV-infected memory CD4$^+$ T cells from uninfected cells.

By examining a selected list of transcripts that differed in their expression patterns between the two types of cell across all study participants, Clark and colleagues identified four genes that were expressed at higher levels in infected than in uninfected cells. These four genes are known to limit the transcription of the HIV genome through the modification of DNA-binding proteins called histones or by pausing the RNA polymerase II enzyme that transcribes genes.

Conversely, the expression of four genes that promote HIV transcription was downregulated in infected compared with uninfected cells. As well as this distinctive pattern of gene expression, which would help the virus to enter a dormant (latent) state, HIV-infected cells exhibited signatures associated with enhanced survival and proliferation. These signatures are reminiscent of those found in memory cells that persist for decades, and which ensure the maintenance of long-lived memory CD4$^+$ T cells[12].

Sun *et al.* focused on the characteristics (phenotype) of HIV reservoir cells, and searched for cell-surface proteins that had different patterns of expression in infected and uninfected cells. Although several previous studies had identified cell-surface proteins that are preferentially expressed by HIV-infected cells[13], that research was limited by the small number of predefined proteins considered or by the need to stimulate cells in which the virus is latent to reveal their infection status. Sun *et al.* developed a single-cell sequencing approach to compare the expression levels of 53 cell-surface proteins in CD4$^+$ T cells from 5 people with HIV who had had no detectable HIV in their blood for years.

HIV-infected cells were identified using a method to amplify 18 small fragments of the HIV genome. This approach enabled the investigators to focus on rare cells that contain genetically intact and potentially replication-competent HIV genomes. It is these cells that should be targeted by strategies aiming to eliminate HIV. However, they are

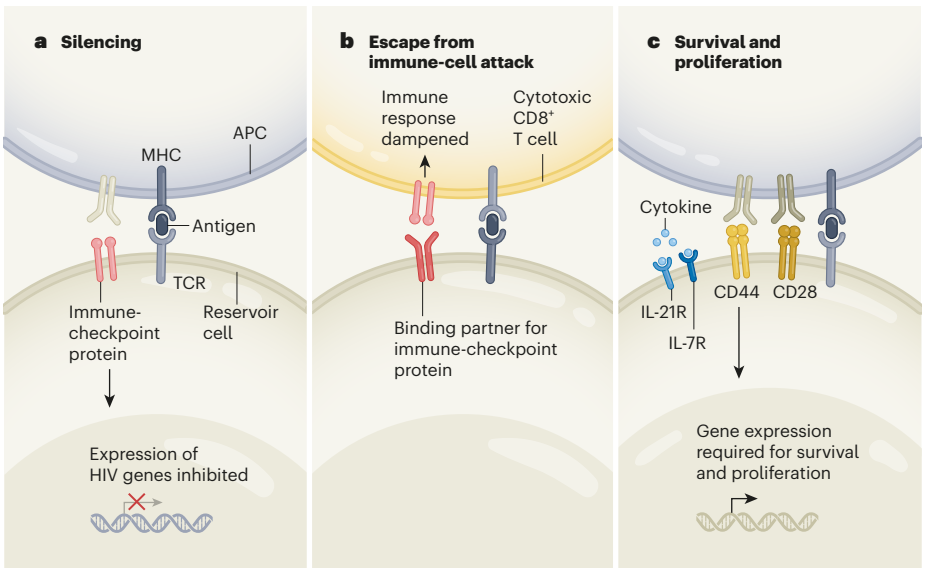

Figure 1 | How HIV-infected cells persist. Immune cells called memory CD4$^+$ T cells that contain HIV integrated into their DNA can persist despite the use of antiviral therapy. Clark *et al.*[5] and Sun *et al.*[6] characterized these reservoir cells, reporting gene-expression signatures and their expression of cell-surface proteins, respectively. **a**, Reservoir cells can be recognized by other immune cells, such as antigen-presenting cells (APCs) and cytotoxic CD8$^+$ T cells. In this process, the T-cell receptor (TCR) binds to peptide fragments called antigens that are displayed by major histocompatibility complex (MHC) molecules. Reservoir cells can engage with various other receptors (beige) on immune cells. Gene-expression analysis identified genes associated with the inhibition of expression of HIV-gene sequences in the infected cells. Such silencing can occur[18,19] when immune-checkpoint proteins are present. **b**, Reservoir cells express binding partners for immune-checkpoint molecules, and interactions between the two types of protein would prevent reservoir-cell destruction by cytotoxic CD8$^+$ T cells. **c**, Reservoir cells display cell-surface proteins (IL-21R, IL-7R, CD44 and CD28) that can bind to APC receptors or signalling molecules called cytokines. Some cell-surface molecules and gene-expression signatures were identified that aid survival and proliferation.

vastly outnumbered by cells with defective viral genomes, which are mostly irrelevant from the perspective of a cure[14].

Compared with uninfected cells, the rare cells likely to contain an intact HIV genome expressed higher levels of 'immune-checkpoint' molecules, which are negative regulators of T-cell function. This finding confirms and extends results from previous studies that reported high expression of immune-checkpoint molecules in reservoir cells[15–17]; such molecules are known to promote HIV latency[18,19]. Notably, HIV-infected cells also expressed several binding partners (ligands) of the immune-checkpoint molecules, suggesting a mechanism by which HIV-infected cells might dampen the killing capacity of immune cells, thereby providing the reservoir cells with a selective advantage in their ability to evade destruction by the immune system.

In contrast to the case with infected cells in the blood, reservoir cells from lymphoid tissue had only a limited number of the proteins that confer protection against immune-cell-mediated killing. Instead, lymphoid reservoir cells showed features associated with resistance to a type of cell death called apoptosis, and signs of expression of molecules that promote survival. This finding showed that the mechanisms responsible for the evolutionary selection of HIV-infected cells differ between the blood and other tissues.

Using distinct but complementary approaches, these two studies reveal features of HIV-infected cells that allow such cells to persist. Because they measure different aspects, the transcriptomic and phenotypic analyses identified different molecules that contribute to the survival of HIV. Nevertheless, the ways in which these molecules promote HIV persistence largely overlapped, suggesting functional redundancy in the underlying mechanisms (Fig. 1).

In both studies, molecules that contribute to HIV transcriptional silencing were found to be more highly expressed in HIV-infected cells than in their uninfected counterparts. Whether the interaction identified by Sun *et al.* between immune-checkpoint molecules and their ligands leads to the upregulation of factors that silence the expression of HIV sequences, as reported by Clark *et al.*, is not known. Also unknown is whether the increased activity of pro-survival and proliferation signalling pathways in HIV-infected cells, as revealed by the gene-expression analysis, is a result of the engagement of the survival-promoting cell-surface proteins indicated by the phenotypic results. Studies combining RNA and protein analyses will be needed to investigate this possibility.

The characteristics of infected cells that have survived years of ART reveal the pressures to which the reservoir is exposed. Given that reservoir cells often express molecules that protect them from being destroyed by immune cells, this points to a mechanism that might be exploited to accelerate the eradication of HIV. For example, blocking immune-checkpoint ligands would make reservoir cells more sensitive to killing by immune cells.

Both studies reveal that the persistence of HIV-infected cells goes beyond the mechanisms that underpin the exquisite capacity of memory $CD4^+$ T cells to endure and to maintain lifelong immunity. Silencing of HIV genes and escape from immune-system pressure are other skills that the long-lived reservoir must use to survive. Whether all three mechanisms must act in each infected cell to ensure its long-term existence remains unclear.

These findings might suggest that we redirect our efforts to eradicate the reservoir, but at what cost? Although it is too early to tell whether all three mechanisms must be targeted simultaneously to eliminate reservoir cells, any approach that would slightly reduce the reservoir but affect the health of people on stable ART would be unacceptable. Unlike the cellular factors that contribute to the longevity of T-cell immunity, which might be difficult to counteract without compromising immunological memory, the cellular and viral molecules that drive HIV latency and escape from destruction by immune cells might represent more-realistic targets for curative strategies.

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Structural biology

Views of a debated transcription complex

Fahad Rashid & James Berger

High-resolution structures of the bacterial Rho protein in complex with an RNA polymerase enzyme and partner proteins provide support for the long-held model of how Rho helps to terminate gene transcription. **See p.367**

Over time, scientific concepts in textbooks can get enshrined into dogma. Occasionally, however, these assumptions are shaken up when new data arise that challenge those cherished models. In 2020, two cryo-electron microscopy studies[1,2] called into question long-standing assumptions about a key facet of bacterial gene regulation known as transcription termination. Now, Molodtsov *et al.*[3] (page 367) describe structures that restore the classical framework. Their results are an elegant demonstration that science sometimes needs to lurch sideways before it can move forwards.

The story begins with a bacterial protein known as Rho, which was discovered[4] in 1969. Not to be confused with an enzyme family of the same name found in nucleus-bearing (eukaryotic) cells, Rho was found to promote the termination of gene transcription — a crucial step in gene regulation that ensures that messenger RNA transcripts are the appropriate length. Over time, several properties of Rho that support this presumed role have been defined. For instance, Rho acts on a large subset of bacterial genes[5]. It forms six-subunit rings that bind to long stretches (more than 50 nucleotides) of RNA[6]. Its sequence is also remarkably similar to