Quiet but not silent: assessing the impact of intact and defective HIV proviral DNA in the brain

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Purpose of review
People with HIV (PWH) require lifelong antiretroviral therapy (ART) to maintain viral suppression, yet integrated HIV DNA persists in long-lived cellular reservoirs in blood and tissues. Most integrated proviruses are defective, meaning they contain deletions, hypermutation, packaging-signal defects, splice defects, or other defects that prevent production of replication-competent viruses. However, these genomes are not necessarily biologically inert as many remain capable of transcription and, in some cases, translation of viral proteins.

Recent findings
Recent evidence has demonstrated that the brain is a stable reservoir of both intact and defective HIV despite viral suppression with ART. Whilst intact replication capable proviruses are known to induce cell activation and pathology, recent studies suggest that defective proviruses with large internal deletions and hypermutation can remain transcriptionally, and in some cases, translationally active and have now been linked with persistent immune activation during ART. Therefore, transcriptionally active defective proviral DNA may be a contributor to underlying neuropathogenesis in ART-suppressed PWH.

Summary
Defective HIV proviruses make up the majority of the proviral reservoir across blood and tissue compartments. Although they are replication-incompetent, many remain transcriptionally and translationally competent and may contribute to chronic inflammation, antigen persistence, and neuropathology in ART-suppressed PWH.

Keywords
intact and defective proviruses, neuroinflammation, reservoirs, transcription

INTRODUCTION

While antiretroviral therapy (ART) suppresses plasma HIV RNA, it does not eradicate integrated proviral HIV DNA. This persistent reservoir remains a barrier to cure and potential contributor to comorbid disease. When ART is interrupted, replication-competent proviruses can rapidly reseed viremia, but the total proviral reservoir is composed predominantly of defective genomes rather than intact, fully replication-competent proviruses [1–3].

A defective provirus is an integrated HIV DNA genome that can no longer complete the full viral life cycle and produce infectious progeny because it carries major defects, such as large internal deletions, APOBEC-mediated hypermutation, premature stop codons, defects in the packaging signal, or disruption of key splice sites [2–5]. Importantly, defective does not mean silent: many defective HIV proviruses retain intact open reading frames, can generate unspliced, spliced or aberrantly spliced RNA, and can express canonical HIV proteins or novel chimeric protein products that may still be sensed by the immune system [4,6,7,8▪▪].

This observation is increasingly important in the context of ongoing comorbidity in ART-suppressed people with HIV (PWH). Current evidence suggests that defective proviruses are not merely inert debris within the reservoir, but a biologically relevant component that can shape immune activation, reservoir

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KEY POINTS

- Across blood and tissue compartments, the HIV proviral reservoir is dominated by defective rather than intact proviruses.
- While defective HIV proviruses are replication-incompetent due to genetic deficits/defects, they may still be transcriptionally or translationally active.
- Defective proviral transcription in tissue reservoirs, including the central nervous system, may contribute to persistent inflammation and neurocognitive impairment despite suppressive antiretroviral therapy.
- Future studies should evaluate both intact and defective reservoirs when interpreting long-term biological outcomes.

measurements, and how treatment responses are understood during ART [4,7,8▪▪,9▪,10].

APPROACHES FOR MEASURING THE INTACT AND DEFECTIVE HIV PROVIRAL RESERVOIR

Precise quantification of the HIV reservoir remains essential for both curative approaches as well as understanding HIV-associated comorbidities, but reservoir assays differ substantially in what they measure and some anatomical sites such as the brain are difficult to measure in living PWH. While near full length sequencing approaches are the gold standard for quantification and characterization of HIV proviral genomes, these are specialized techniques requiring large sample input and significant bioinformatic analysis. In compartments with restricted tissue access, two-target assays such as the intact proviral DNA assay (IPDA) have proven to be scalable and useful for high-throughput studies, yet sequence heterogeneity and limited genomic coverage can lead to quantification failures or misclassification of defective proviruses as intact [1,11–13,14▪▪]. Newer multitarget approaches have addressed this potential limitation. Q4ddPCR simultaneously targets four conserved regions of the HIV genome and closely matches sequence-confirmed intact reservoir measurements. Across multiple cohorts, the Q4ddPCR assay quantifies intact proviruses in most tested samples, correlates strongly with viral outgrowth, and reports lower intact proviral frequencies and a steeper early decline on ART than IPDA [14▪▪]. These findings support the view that multiprobe assays provide a more accurate estimate of intact reservoir dynamics, whereas simpler assays remain useful for total HIV DNA but should be interpreted cautiously when estimating intact reservoirs [1,11–13,14▪▪]. These limitations may be particularly relevant in tissue reservoirs, including the central nervous system (CNS), where proviral composition and transcriptional activity remain incompletely characterized and very difficult to access.

TISSUE AND CELL-SPECIFIC PREVALENCE OF DEFECTIVE HIV PROVIRUSES

Full-length sequencing and PCR-based studies consistently show that defective HIV proviruses account for 80–90% of HIV proviral DNA across peripheral blood and tissue compartments, including gut, lymphoid tissue, genital tract, liver, cerebrospinal fluid, and the brain [3,15–22] (Table 1). This is consistent across compartments and highlights that reservoir composition and activity, not just reservoir size, are critical when interpreting HIV persistence during ART [1,2,3].

The major unresolved question is no longer whether defective proviruses are common, but how their composition differs by anatomical site and cell type and whether these differences matter biologically [23–25]. This is especially relevant in tissues enriched for macrophages or tissue-resident myeloid cells such as the brain where local transcriptional programs and immune sensing may differ from those in circulating $CD4^+$ T cells [15–17,20,25].

PROPOSED MECHANISMS OF THE GENERATION OF DEFECTIVE HIV PROVIRUSES

Defective HIV proviruses arise through a combination of viral replication errors and host restriction mechanisms operating during reverse transcription. Among the best-characterized pathways are the generation of large internal deletions, restriction of reverse transcription by SAM and HD domain-containing protein 1 (SAMHD1), aberrant template switching and strand transfer, and apolipoprotein B mRNA editing enzyme, catalytic subunit 3 (APOBEC3)-mediated hypermutation. Together, these processes contribute to the heterogeneous composition of the defective proviral reservoir [3,23,26–39]. Large internal deletions are among the most common defects in HIV proviral DNA and arise during reverse transcription when synthesis is interrupted, and nonadjacent regions of the viral genome are joined together. Analyses of deletion junctions and recent mechanistic studies support a model in which these defects reflect aberrant strand-transfer events rather than random DNA damage alone [3,32,34]. SAMHD1 restricts replication by lowering intracellular deoxyribonucleotide triphosphate (dNTP) availability, particularly in resting $CD4^+$ T cells and

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Table 1. Summary of studies characterizing intact and defective HIV-1 proviral DNA across CNS compartments relative to peripheral compartments in ART-suppressed PWH

Sample	Cohort	Cell type	FLS	PCR		Intact proviruses (%)	Defective proviruses (%)	Citation
				Assay	Targets			
PBMC	ART-treated, $n=42$	CD4$^+$ T cells	N	Q4ddPCR	Psi, Env, Gag, Po	6%	94%	Scheck *et al. Nat Commun*, 2026 [14▪▪]
PBMC	ART-suppressed, $n=19$ (2016) ART-suppressed, $n=57$ (2019)	CD4$^+$ T cells	Y	IPDA	IPDA: Psi, Env	2.4%	97.6%	Bruner *et al. Nature,* 2019 [1] Bruner *et al. Nat Med,* 2016 [3]
Brain (Hippocampus, BG, FL)	ART-suppressed, $n=2$	Whole tissue	N	Gag, Alu-PCR	Gag, integrated proviral DNA	n/a – intact detected from outgrowth assay		Tang *et al. J Clin Invest,* 2023[55]
Brain (BG, OCC, FL, PVWM; thalamus)	ART-treated, $n=3$ (2/3 ART-suppressed)	Whole tissue	Y	IPDA	IPDA: Psi, Env;	Not reported as #	Not reported as #	Sun *et al. eLife*, 2023 [20]
Brain (FL), lymphoid tissue (spleen, lymph node, GALT)	ART-suppressed, $n=10$	Whole tissue	N	IPDA, Pol	IPDA: Psi, Env; Pol	11%	89%	Cochrane *et al. Ann Neurol*, 2022 [15]
Brain (FL, BG, cerebellum)	ART-suppressed, $n=8$	Whole tissue	N	IPDA	IPDA: Psi, Env;	12%	88%	Angelovich *et al. Ann Neurol,* 2023 [16]
Brain (FL)	ART-suppressed, $n=28$	Whole tissue	N	IPDA, Gag	IPDA: Psi, Env; Gag	7%	93%	Gabuzda *et al. Viruses,* 2023 [17]
PBMC, CSF	ART-treated, $n=11$ (7/11 ART-suppressed)	Pooled	N	IPDA	IPDA: Psi, Env;	1.7%	97.25%	Zhang *et al. Neurotherapeutics,* 2024 [61]
Brain (FL, OCC, SVZ)	ART-treated, $n=5$ (2/5 ART-suppressed)	Whole tissue	N	LTR, IPDA	LTR; IPDA: Psi, Env	8.4%	91.6%	Nuhn *et al. Viruses,* 2025 [56▪▪]

BG, basal ganglia; CD4$^+$T cells, cluster of differentiation 4$^+$ T lymphocytes; CSF, cerebrospinal fluid; CTLA-4, cytotoxic T lymphocyte associated protein 4; FL, frontal lobe; nFLS, near full length sequencing; GALT, gut associated lymphoid tissue; IPDA, intact proviral DNA assay; n/a, not applicable; OCC, occipital lobe; PBMC, peripheral blood mononuclear cells; PD-1, programmed cell death protein 1; PVWM, periventricular white matter; Q4ddPCR, Q4 droplet digital polymerase chain reaction; RU5, R and U5 untranslated region of HIV; SVZ, subventricular zone.

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myeloid cells [28,29,31,33,35,37,40]. By stalling or destabilizing reverse transcription, SAMHD1 activity may indirectly promote incomplete DNA synthesis and thereby contribute indirectly to the generation of defective proviruses, although the precise link to specific defect classes remains unresolved.

Template switching and strand transfer are normal features of HIV reverse transcription, but when this mechanism fails or is inaccurate, it can generate internal deletions and other defective genomes. This mechanism is now strongly supported by sequence-based studies showing recurrent deletion hotspots and by recent models proposing multiple aberrant transfer pathways across both minus-and plus-strand synthesis [3,32,38,41–43]. APOBEC3 proteins promote another major class of defect by deaminating viral DNA during reverse transcription, leading to G-to-A hypermutation and loss of replication competence. Although these hypermutated proviruses are defective, some may still be transcribed, making them relevant to reservoir biology even when they cannot produce infectious virus or canonical viral proteins [4,27,30].

FUNCTIONAL ACTIVITY OF DEFECTIVE HIV PROVIRUSES

Defective proviruses can remain transcriptionally active despite lacking the capacity to generate infectious virus; a concept established by mechanistic studies of splicing, translation, and cytotoxic T cell recognition from defective genomes [4,6,7,10]. Transcription from defective HIV proviruses has been observed across multiple defect classes, including proviruses with defects in the packaging signal, the major splice donor, hypermutations, large internal deletions, and nonsense mutations, and this activity is detected in both viremic and ART-suppressed settings [4,6,10]. In many cases, the sequence of cell-associated HIV RNA closely mirrors that of the underlying defective provirus, supporting the view that a substantial fraction of persistent HIV transcripts during ART arise from archived defective genomes rather than from ongoing cycles of replication [4,6,7,10].

The transcriptional products of defective proviruses include both spliced and unspliced HIV RNA [4,6,8▪▪,10]. Splicing can still occur even when the major splice donor is altered or deleted, with activation of alternative splice sites generating aberrant but protein-coding transcripts [4]. Other defective proviruses give rise to unspliced transcripts in which neither canonical nor clear cryptic splice donor or acceptor sites are identifiable, highlighting the flexibility of HIV RNA processing within the defective reservoir [4,6,10]. Additional work has shown that intragenic regulatory elements can initiate transcription from within the viral genome, generating 5′ untranslated regions (UTR)-deficient *env* and *nef* transcripts even when the long terminal repeats (LTR) is compromised, further expanding the range of RNAs that can be produced from defective proviruses [4,7,44].

These RNAs are not necessarily biologically inert. Truncated defective HIV proviruses can preserve translationally competent open reading frames for canonical HIV proteins as well as novel chimeric proteins, and protein expression has been demonstrated when coding capacity for viral proteins such as Gag and Nef is retained [4,6,7]. Cells harboring such defective proviruses can present viral epitopes and be recognized by HIV-specific cytotoxic T lymphocytes, indicating that expression from defective genomes can shape the proviral landscape by immune-mediated clearance of specific clones [4]. Together, these observations extend the significance of defective proviruses beyond transcription alone and support the idea that the defective reservoir contributes to persistent antigen exposure during ART [4,7,8▪▪,10].

Serological and biomarker data are consistent with ongoing antigenic stimulation from defective HIV proviruses. Longitudinal and cross-sectional analyses show persistent antibodies to HIV proteins despite durable virologic suppression, and these antibody responses correlate with transcription from defective proviruses and with markers of immune activation in ART-treated cohorts [8▪▪,10,45]. Elevated inflammatory and coagulation markers, including IL-6, high-sensitivity C-reactive protein, tissue factor, and D-dimer, have been reported in people on long-term ART, supporting a link between low-level HIV expression and chronic immune stimulation [8▪▪,10]. Recent *in vitro* studies have also shown that intron-containing and other aberrant HIV RNAs derived from proviral DNA can trigger type I interferon and inflammatory cytokine production, providing a mechanistic route by which transcriptionally active defective proviruses could sustain innate immune activation [46,47]. However, the precise contribution of defective HIV proviruses to systemic inflammation remains unclear and an important question to be answered.

Persistent HIV transcription is observed not only in blood but also across tissue reservoirs, including gut and brain, where levels of HIV transcription as well as transcriptional blocks differ from those in circulating $CD4^+$ T cells [5,19,26,48–51]. However, most tissue-based transcription studies cannot fully disentangle RNA arising from intact proviruses from RNA arising from defective proviruses, particularly truncated genomes [5,48–51]. Consequently, the precise contribution of defective proviral transcription to persistent inflammation in different tissue sites remains incompletely defined, even though

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proviral DNA and HIV RNA in the CNS have been associated with ongoing neuroinflammation in virally suppressed people with HIV [9▪,48].

Taken together, these findings support a view of the defective HIV reservoir as a persistent source of HIV RNA, protein expression, and immune stimulation rather than inert genomic 'junk' [1,4,6,7,8▪▪, 13,52]. This reinforces the need for cure-directed studies and reservoir assays to consider not only replication competence but also the transcriptional and translational potential of defective proviruses across blood and tissue compartments when interpreting long-term outcomes under ART.

IMPACT OF INTACT AND DEFECTIVE HIV PROVIRUSES ON THE BRAIN

The central nervous system (CNS) represents a unique anatomical HIV reservoir because it contains long-lived tissue resident myeloid populations, including microglia and perivascular macrophages, that can persist for prolonged periods despite suppressive ART [9▪,15–17,20,48,53,54]. HIV enters the brain early during infection and integrated proviral DNA persists even in individuals with durable plasma viral suppression, supporting the view that the CNS remains an important tissue reservoir during long-term ART. We and others have quantified the level of both intact and defective HIV proviral DNA in autopsy frontal white matter brain tissue [9▪,15,17,48] (Table 1). Importantly, the levels of both intact and defective HIV proviral DNA were comparable between ART-suppressed PWH and nonsuppressed PWH, supporting the persistence of a stable HIV reservoir in the brain. We further identified that the level of both intact and defective HIV proviral DNA was higher in the frontal cortex relative to basal ganglia or cerebellum [16]. Near full length sequencing analysis of the brain has also detected intact proviral DNA in some PWH treated with ART [20]. Importantly, microglia isolated from brain tissue following rapid autopsy have been shown to produce replication competent HIV capable of infecting cells *ex vivo*[55,56▪▪]. However, although intact replication competent HIV has been detected in the brain, current evidence suggests that defective HIV proviruses comprise the majority of proviral DNA within CNS tissue, consistent with observations in blood and other anatomical reservoirs [3,15–17,20].

Recent studies demonstrate that HIV transcription also persists in the brain and CSF despite suppressive ART [48,57–59]. However, whether these transcripts predominantly arise from intact or defective proviruses remains unresolved because most tissue-based approaches cannot fully distinguish transcription originating from replication competent genomes or defective proviruses, particularly truncated genomes retaining transcriptionally active regions [5,26,48–51,60]. This distinction is biologically important because defective proviruses may continue to produce viral RNA species, viral proteins, or aberrant transcripts capable of activating innate immune sensing pathways despite lacking replication competence.

Persistent neuroinflammation remains a common feature of HIV infection despite ART and has been associated with both HIV DNA and HIV RNA within the CNS [9▪,48]. We have found that intact and 5′ defective HIV proviral DNA (as measured by IPDA) were associated with Mx1+microglia in the frontal white matter of ART-suppressed PWH [9▪]. Gabuzda *et al.* also identified a positive association between the level of intact HIV proviral DNA and gene expression of measures of inflammation and cell stress [17], although no association was reported for the level of defective HIV proviruses as measured by IPDA. Interestingly, PWH with neurocognitive impairment did not have different levels of intact HIV DNA relative to those without detectable levels. Transcriptionally active defective proviruses may contribute to this inflammatory environment through ongoing production of intron-containing HIV RNA and other aberrant transcripts that can activate type I interferon and inflammatory cytokine pathways [47]. This mechanism may be particularly relevant in the CNS, where chronic activation of microglia and infiltrating myeloid cells is closely linked with neuronal injury and persistent neurocognitive impairment in people with HIV despite viral suppression [9▪,48]. In this context, defective proviruses may represent a sustained source of inflammatory stimulation even in the absence of productive viral replication.

An additional unresolved question is whether defective proviral landscapes differ between circulating $CD4^+$ T cells and CNS-resident myeloid populations. The CNS has distinct cellular composition, immune surveillance, and transcriptional environments that may influence proviral persistence, transcriptional activity, and immune recognition differently than in peripheral blood [15–17,20,24,25,48, 50]. Regional heterogeneity within the brain may also be important, as we demonstrated differences in intact and defective proviral frequencies across anatomical brain regions in both viremic and virally suppressed PWH [16]. Defining whether specific classes of defective proviruses preferentially persist or remain transcriptionally active within CNS myeloid reservoirs will therefore be important for understanding persistent neuroinflammation during ART.

Together, these findings support the concept that defective proviruses within the CNS are unlikely to represent biologically inert genomic remnants. Instead, they may contribute to persistent HIV

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transcription, chronic innate immune activation, and ongoing neuroinflammatory signaling despite suppressive ART. Further studies integrating intactness assays with spatial and single-cell transcriptomic approaches will be required to define the contribution of defective proviruses to CNS reservoir biology and HIV-associated neuroinflammation.

CONCLUSION

The modern view of the HIV reservoir is that defective HIV proviruses are not simply inert genomic remnants, but dynamic and biologically relevant components of HIV persistence. They constitute the majority of proviral DNA, can remain transcriptionally and sometimes translationally active, and are increasingly implicated in chronic immune activation and incomplete immune recovery during suppressive ART which poses significant implications for the brain. For this reason, HIV treatment strategies should assess how interventions affect both intact and defective HIV proviral populations. A reduction in intact HIV DNA may predict reduced rebound risk, but persistent defective proviruses may still matter biologically if they continue to generate RNA, proteins, or inflammatory signaling that contribute to comorbid disease. Future management of HIV may therefore need to suppress not only replication-competent viruses, but also the transcriptional and immunostimulatory activity of defective proviruses.

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Conflicts of interest

There are no conflicts of interest.

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