

Intragenic transcription from defective HIV proviruses triggers interferon responses in myeloid cells

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ABSTRACT The persistent HIV-1 reservoir includes a subset of cells harboring transcriptionally repressed latent HIV-1 that contributes to rebound upon antiretroviral treatment (ART) interruption. However, the majority of the reservoir consists of defective proviral genomes with mutations that prevent production of HIV-1 virions. People with HIV (PWH), even with suppression of viremia, demonstrate comorbidities of the central nervous system, heart, gut, and general aging-associated inflammation. Previously, we identified a transcriptionally active element within the envelope gene (*env*) of HIV-1, which mediates the expression of aberrant HIV-1 RNAs. We hypothesize that spurious expression of defective proviruses contributes to the general inflammation that drives these comorbidities. We observed correlations between levels of inflammatory cytokines in serum of PWH and levels of HIV-1 transcripts from this intragenic promoter. To investigate the impact of defective proviruses, we employed CRISPR-Cas9 to render the 5' long terminal repeat (LTR), which acts as the enhancer and promoter for proviral transcription, non-functional. HIV-1-infected cells harboring this deletion produce significantly higher levels of IP-10 and IL-8 *in vitro* in both monocytic cell lines and primary monocyte-derived macrophages. Transcripts generated from the *env* promoter include a 5' cap and polyA tail, and the induction of IP-10 expression was dependent on the cytosolic innate immune sensing pathway components MDA5 and MAVS and not cGAS and RIG-I. We propose that defective HIV proviruses contribute to chronic inflammation in PWH through an MDA5-dependent induction of type I interferon pathways.

IMPORTANCE People with HIV-1 are at higher risk of developing age-associated comorbidities and immune exhaustion even when receiving antiviral treatments and having no detectable viremia. Transcription and translation have been documented from latent and defective proviruses, but their impact on inflammation associated with chronic HIV-1 infection remains poorly understood. The significance of this work is in identifying a role for defective HIV-1 proviruses and correlating their transcription in triggering a type I interferon response. These results highlight the importance of the persistent defective HIV-1 proviruses and understanding their impact on driving chronic inflammation to inform future strategies to assure people with HIV-1 healthy living and aging.

KEYWORDS HIV-1, defective proviruses, transcription, inflammation

Human immunodeficiency virus (HIV) remains a global burden even with the availability of effective antiretroviral therapies (ART) (1). Recent estimates put the number of people with HIV (PWH) worldwide at approximately 40 million, with an annual new infection rate of 1.3 million per year (2). Upon infection, HIV-1 integrates a DNA copy of its genome into the host cell's DNA, establishing a provirus that persists for

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the life of the cell (3, 4). Transcription of a subset of these proviruses is repressed, and in the context of ART, it will evade targeting by the immune response to generate a reservoir of infected cells (5–8). These latently infected cells, which include CD4⁺ T cells, macrophages, dendritic cells, and microglia, present the primary barrier to a cure since they support new virus replication and infection following treatment interruption (9–11). Low levels of cell-associated HIV-1 RNA have been identified in peripheral blood mononuclear cells (PBMCs) from PWH on ART, even when viral load is below the limit of detection (LOD) (12–14). While ART prevents the spread of HIV, it is not a cure (15, 16).

PWH are at higher risk of developing comorbidities including neurological deficits, frailty, cardiovascular disease, gut dysbiosis, and a general accelerated aging, often referred to as “inflammaging” (17–20). This increased susceptibility to inflammatory diseases persists in PWH actively being treated with ART as well as controllers who repress HIV-1 in the absence of ART, indicating that these chronic comorbidities are not solely driven by active HIV-1 replication (21–25). It has been proposed that spurious reactivation of the latent reservoir or blips, ART interruption leading to rebound, and long-term immune cell exhaustion and dysregulation of inflammation contribute to HIV-1-associated inflammation (26, 27).

Recent work has identified multiple pathways that initiate HIV-1-mediated inflammation. For example, CARD8 inflammasome activation by HIV-1 protease and NLRP1 inflammasomes triggered by unspliced HIV-1 RNA induce the production of IL-1 β and pyroptosis in infected cells (28, 29). In addition, expression of peptides during latent infection has been shown to lead to antigen presentation and shaping of T cell responses (14, 27). Furthermore, unspliced intron-containing HIV-1 RNAs (icRNAs) induce type I interferon (IFN) responses in myeloid cells through an MAVS-dependent pathway (30, 31). Importantly, the majority of persistent HIV-1 genomes are defective proviruses that harbor deletions, insertions, inversions, mutations, and other errors that prevent the production of new virions; however, these defective proviruses are transcriptionally and translationally active, producing HIV-1 RNAs and proteins that potentially perpetuate inflammation (5, 6, 13, 32). Therefore, HIV-1 generates a variety of pathogen-associated molecular patterns (PAMPs) that are detected by pattern recognition receptors (PRRs) to initiate general inflammation (29, 33–37).

We previously identified a transcriptional element within the HIV-1 *env* gene that supports transcription even in the absence of a functional 5′ long terminal repeat (LTR) (38). Transcripts driven by this intragenic HIV-1 promoter have been detected in PBMCs from PWH on ART (26, 38). We hypothesize that active transcription from defective proviruses induces innate immune responses and thus contributes to the persistent inflammation and immune dysregulation observed in PWH on ART. In this study, we show that intragenic transcription associated with defective proviruses correlates with induction of IFN type I responses in clinical samples, myeloid cell lines, and primary macrophages. Furthermore, RNA-mediated IFN I response is dependent on the MDA5 signaling pathway. We propose a model that innate immune sensing of spurious RNAs from defective HIV-1 proviral genomes perpetuates inflammation in PWH.

RESULTS

Cell-associated UTR-deficient transcripts in PWH correlate with increased serum cytokine levels

Our hypothesis is that spurious transcription from defective HIV-1 proviruses induces inflammation. We explored whether there was a correlation between levels of cytokines in PWH and specific subsets of HIV-1 transcripts, those initiated at the LTR and those noncanonical RNAs that were transcribed from intragenic elements. We examined data collected from 22 PWH in a previously described “inflammaging” cohort consisting of PWH on ART for at least 6 months with no detectable viremia (Table 1) (39). Additional demographic information, including CD4⁺ T cell count at time of sample collection and sex assigned at birth, is also included in Table 1. As part of the original study, cytokine levels were assessed by enzyme-linked immunoassay (ELISA) (39). We focused on those

TABLE 1 Cohort description

Characteristic	All PWH (<i>n</i> = 22)
Age, years, median (range)	52 (26–68)
Female sex, no. (%)	8 (36.4)
Duration of therapy, years, median (range)	11.49 (2.38–20.63)
Absolute CD4 count (cells/mm ³), median (range)	638 (194–1,523)

cytokines that had a range of concentrations above the limit of detection, specifically TNF- α , IP-10, IL-6, IL-8, and IL-12p70. RNA was isolated from PBMCs for these 22 samples, and multiplex-RTddPCR (Fig. 1A and B) was used to quantify levels of total HIV RNAs, 5' untranslated region (UTR)-containing RNA initiating from the LTR, and UTR-deficient, *env*-positive RNA arising from alternative promoter-like elements. Univariate analysis for each RNA subset, total RNA, each cytokine, and the demographic factors was run to identify notable correlations (Fig. 1C). UTR-deficient RNAs correlated significantly with the serum levels of IP-10, TNF- α , and IL-8. Total HIV-associated RNA did not correlate strongly with any cytokines, nor did UTR-containing RNA (Fig. 1C). Based on these analyses, we ran multiple linear regressions for combinations of HIV-1 RNA populations and these three cytokines, while adjusting for age, biological sex, and time on ART (Fig. 1D through F). Age, time on ART, and biological sex did not affect the correlations between UTR-deficient RNAs and TNF- α , IP-10, or IL-8 (Table S5 and S7). As shown in Fig. 1C through F, there is a correlation between UTR-deficient RNAs and these cytokines.

Defective provirus triggers IP-10 and IL-8 production *in vitro*

Previous work from our lab identified an intragenic promoter-like element capable of supporting transcription from defective proviruses (38). To explore whether transcription from this intragenic element contributes to inflammation, we used CRISPR to generate defective proviruses that lacked LTRs in infected cells. As proof of concept for our general strategy, we used a Jurkat cell line engineered to constitutively express Cas9. Following infection of these cells, we transfected plasmids that expressed specific guide RNAs (gRNAs) targeting the integrated provirus, deleting approximately 1 kilobase of proviral genome from the 5' LTR to the 3' end of *gag* (Fig. 2A). The 5' LTR deletion was confirmed by PCR using primers that flanked the indel sites (Fig. 2B). The PCR amplicons were analyzed by gel electrophoresis (Fig. 2C and Fig. S1) and sequenced by Nanopore sequencing (Fig. S2). The number of reads that mapped to either the full-length fragment (1.5 kb) or the modified fragment (0.4 kb) was used to estimate the percent of conversion from intact to defective provirus. We detected a 400-bp fragment in infected Jurkats treated with specific guides and observed a decrease in the intensity of the band associated with intact HIV-1 provirus (Fig. 2C and Fig. S1). Sequencing showed that approximately 25% of the reads corresponded to modified proviruses (Fig. S2). Production of interferons from the infected Jurkat cells was assessed by adding spent media from the Jurkat-HIV-1-infected cells to a HEK-293-ISRE-Luc cell line, which includes an interferon stimulation response element driving a luciferase reporter; addition of type I IFNs to this cell line induces luciferase expression (40). A modest three-fold induction with supernatants from cells harboring HIV-1 genomes modified by CRISPR was observed. Controls including mock-infected cells and cells treated with scrambled RNAs, as well as cells infected with an HIV-1 clone in which *env* was disrupted by GFP, did not induce an interferon response (Fig. 2D). These results suggest that defective proviruses contribute to a type I IFN response.

HIV-1-mediated inflammation and comorbidities have been associated with myeloid cell and macrophage dysfunction, including dysregulated production of type I IFNs. To examine whether defective HIV-1 proviruses alter macrophage activity, we utilized CRISPR-Cas9 ribonucleoproteins (RNPs) to generate monocyte-derived macrophages (MDMs) harboring defective proviruses. Generation of defective proviruses was confirmed by PCR as described above (Fig. S1D through F and Fig. S2B). Nanopore

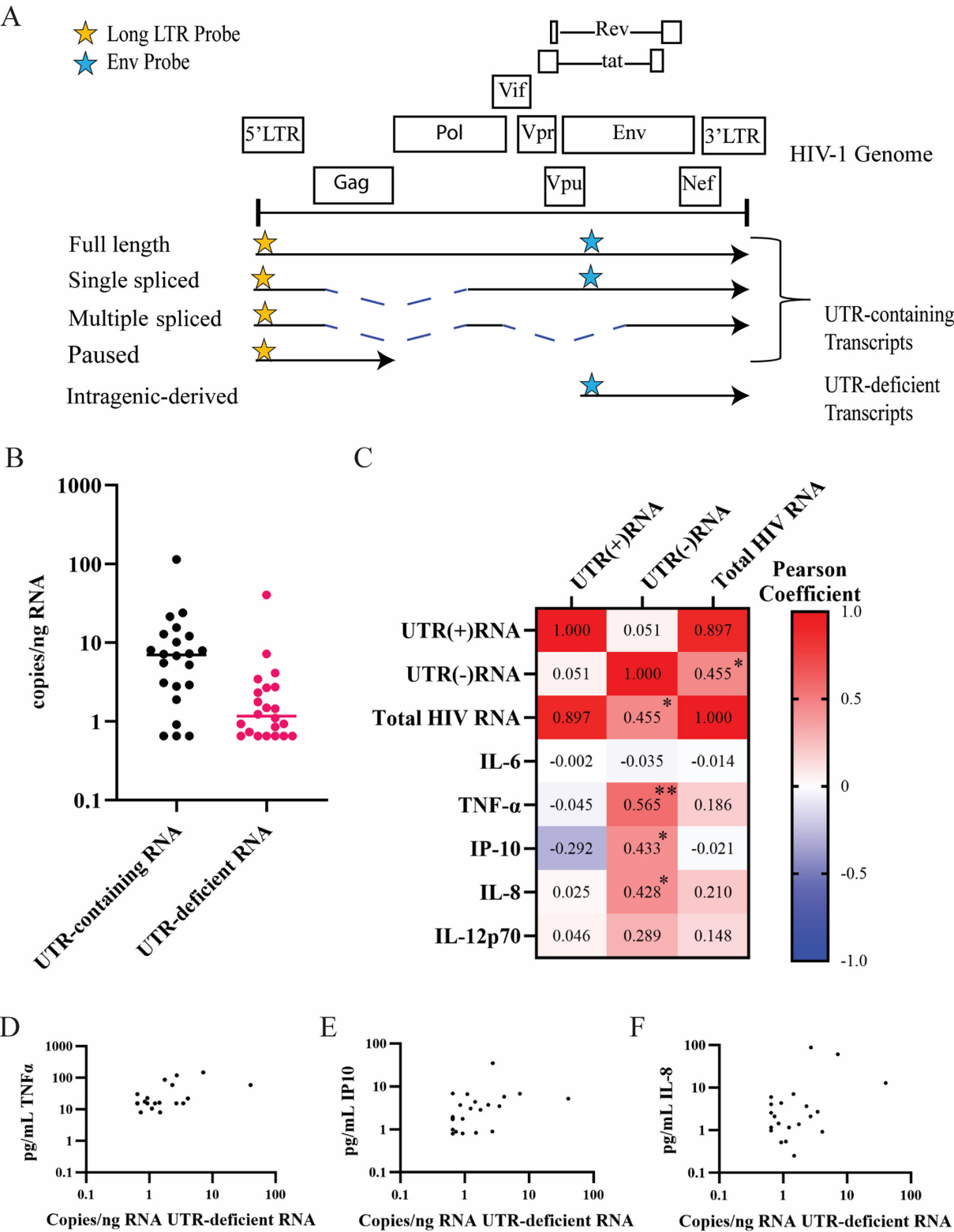


FIG 1 Expression of inflammatory cytokines correlates with UTR-deficient HIV RNA. (A) Diagram of the multiplexed RTddPCR assay used to measure HIV-1 transcripts with or without the UTR sequence. Adapted from Kilroy et al., 2021 (26). (B) Quantification of HIV UTR-containing and UTR-deficient RNAs from 22 PWH. (C) Heat map showing Pearson correlation coefficients between UTR-containing RNA, UTR-deficient RNA, or total HIV-1 RNA modeled against serum cytokine levels. The log of each raw value was taken prior to correlation tests. * represents *P*-values below 0.05, and ** represents *P*-values below 0.01 for the univariate analysis. (D–F) XY scatter plots of cytokines TNF- α (D), IP-10 (E), and IL-8 (F), which were significantly correlated with UTR-deficient RNAs. (Continued on next page)

Fig 1 (Continued)

Untransformed measures of cytokines (pg/mL) and UTR-deficient RNA (copies/ng RNA) are plotted. Axes are on a log-10 scale. Multiple linear regression shows significant correlations between UTR-deficient RNAs and IP-10 ($P = 0.0353$, Table S5), TNF- α ($P = 0.0213$ Table S6), and IL-8 ($P = 0.0375$ Table S7).

sequencing suggested approximately a 40% conversion of intact to defective proviruses in MDMs (Fig. S2B). HIV-1 DNA levels were determined with an *env* qPCR using a pNL4-3 standard, and there was no significant difference in infection between scrambled and 5' deleted conditions (Fig. S3A). RT-qPCR was performed to examine the transcriptional induction of inflammatory cytokines and interferon-stimulated genes (ISGs). We observed significant increases in IP-10 and IL-8 transcription in cells harboring modified provirus when compared to expression from unmodified provirus controls (Fig. 3A and C). TNF- α mRNA was detected in all conditions, but with no significant difference between mock-infected, scrambled RNP controls, or modified proviruses (Fig. 3D). Transcription of IFN2 α , IFN- γ , and IL-6 was below the limit of detection in all samples.

Induction of IP-10 protein and transcription was the most robust response observed when comparing cells with modified or unmodified control HIV-1 proviruses. IP-10 transcription increased 75 to 1,000-fold, while IP-10 protein production increased 3 to 4-fold (Fig. 3A and B). IRF1 transcription levels were unchanged between conditions, which is expected since this interferon-stimulated gene is regulated by post-translational modification (Fig. 3E) (41–43). Controls, including mock infection, efavirenz (EFV)-treated infection, scrambled gRNA controls, and CRISPR modification to the human gene *hprt*, did not induce IP-10 when compared to untransfected cells (Fig. S4A and B), with the latter indicating that CRISPR-Cas9 targeting of an endogenous gene does not induce an interferon response. Taken together, these data support the hypothesis that defective HIV-1 proviruses contribute to myeloid cell dysfunction and inflammatory activity.

Characterization of UTR-deficient RNA

To characterize the nature of the RNAs generated from defective proviruses, we used bulk RNA from MDM or wild-type THP1 cells harboring defective proviruses. To determine if the RNAs contained a 5'-methylguanosine cap, typically associated with mRNAs, we used recombinant eIF4e protein conjugated to GFP and pulled down 5' capped RNAs using anti-GFP capture beads. Pull-down UTR+ and UTR-deficient RNAs were assessed by RTddPCR and compared to bulk RNA from the original population. UTR-deficient RNAs were pulled down by eIF4e in both PMA-differentiated THP1s (Fig. 4A and Fig. S5A and B) and MDMs (Fig. 4C and Fig. S5C and D), suggesting that they are 5' capped. Some variation in HIV-1 infection and RNA expression between replicates from THP1s and within donors from MDMs was observed, but UTR-deficient RNAs were detected after pulldown in all experiments. The specificity of the pulldown was assessed by comparing the levels of leucine-tRNA measured with qPCR in the pre- and post-pull-down RNA populations since tRNAs do not have a 5'-methylguanosine cap (Fig. 4B and D). We did not detect significant levels of leucine tRNA following 5' cap pulldown, although we did detect RPL13a mRNA, a housekeeping gene that is 5' capped (Sup Fig. S6A and B). To determine whether these RNAs are polyadenylated, we generated cDNA using either polydT primers or a combination of random hexamers and polydT primers and compared the ability to amplify UTR-containing and UTR-deficient HIV-1 RNAs using multiplex RTddPCR. We observed intragenic-derived RNAs in both samples (Fig. 4E and F), consistent with the noncanonical RNAs having a 3' polyA tail, as observed in our previous study (38). Overall, these data indicate that intragenic-derived transcripts are modified with a 5' cap and polyA tail.

MDA5 and MAVS are necessary for innate immune responses to defective HIV-1 proviruses

RNAs can act as PAMPs that are recognized by intracellular PRRs. For example, MDA5 has been shown to recognize double-stranded RNAs. Furthermore, the MDA5-MAVS

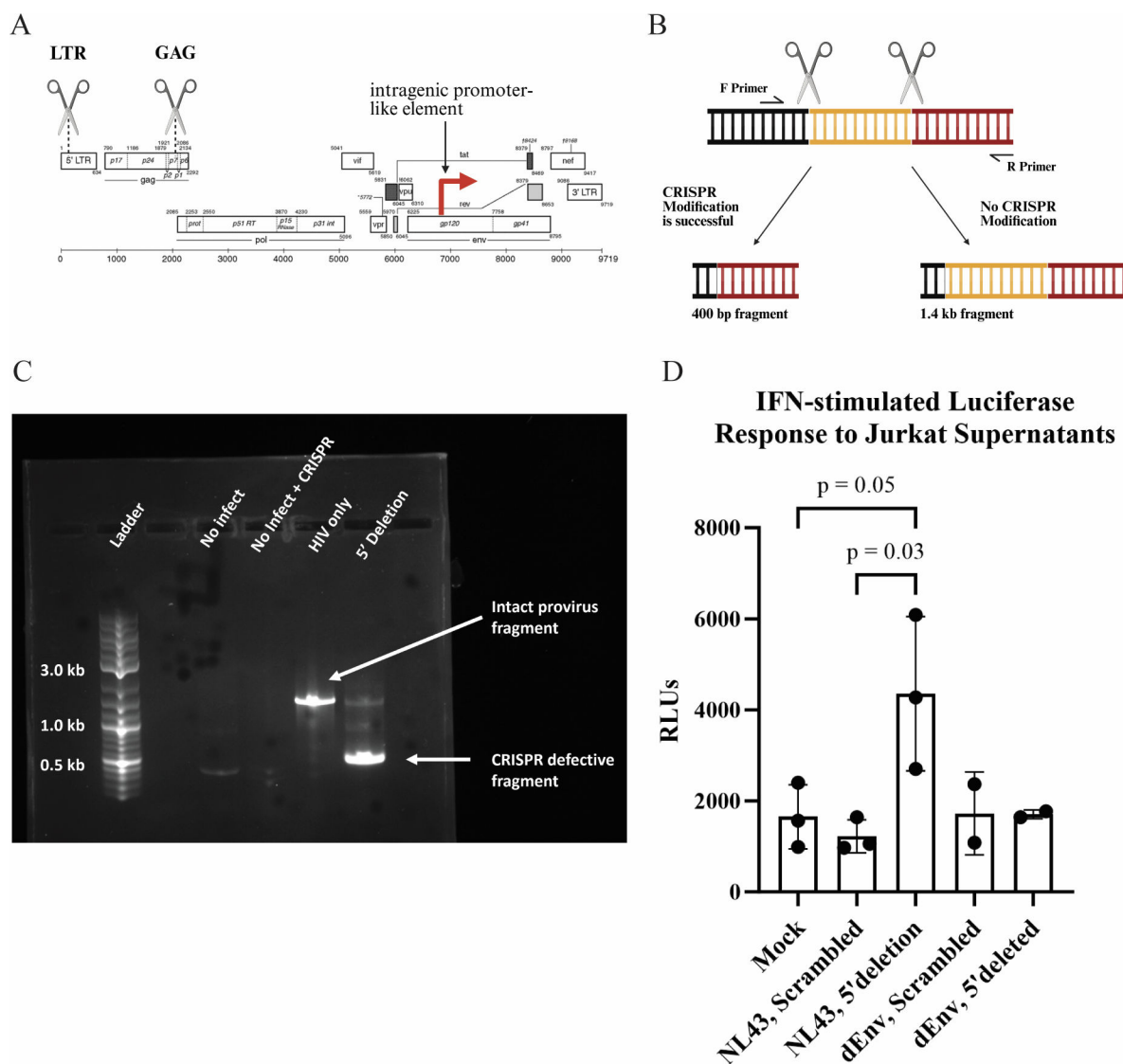


FIG 2 Engineered defective HIV-1 proviruses in Jurkat cells induce type I IFNs. Jurkat-Cas9 cells were infected with VSVg-pseudotyped NL4-3 or VSVg-pseudotyped NL4-3-dEnv-GFP at a multiplicity of infection (MOI) of 0.2. CRISPR gRNA-expressing plasmids were nucleofected 24 h post-infection. DNA and supernatants were collected 48 h post-infection. (A) Map of the HIV-1 genome annotated with the CRISPR-Cas9 target sites and the intragenic promoter-like element. (B) Diagram of the PCR strategy for confirming CRISPR deletion of the HIV-1 genome. (C) Agarose gel with PCR products confirming CRISPR modification of HIV-1 provirus in Jurkat cells. (D) HEK-293-ISRE-Luc cells were treated with supernatants from Jurkat cells harboring defective proviruses and compared to similarly modified NL4-3-dEnv-GFP virus in which the intragenic element is disrupted. Higher RLUs correspond to higher concentrations of interferons in supernatants. There are three replicates for the mock condition and each condition infected with NL4-3 and two replicates for each condition infected with NL4-3-dEnv-GFP. Error bars represent standard error, and analysis of variance (ANOVA) and paired Student's t-tests following a Tukey's correction. *P*-values are shown.

signaling cascade has been shown to be necessary for detection and induction of type I IFN responses to HIV-1 intron-containing RNAs (29–31). To determine if the MDA5-MAVS pathway was similarly necessary for the type I IFN response triggered by defective HIV-1 proviruses, we utilized THP1 cell lines that had MDA5, MAVS, cGAS, or RIG-I knocked down (37). THP-1 cells were infected with HIV-1 and treated with RNPs with Cas9 and guide RNAs, as described above. Knockdown was confirmed with PCR (Fig. S1G through I). In wild-type THP1s, an NL4-3-dEnv-GFP virus was used as a control for CRISPR modification in the absence of the intragenic promoter-like element (Fig. 5A). CRISPR-Cas9 knockdown efficacy was determined as before. Sequencing of proviral DNA suggests approximately 21% modification was achieved in THP1s (Fig. S2C). Controls

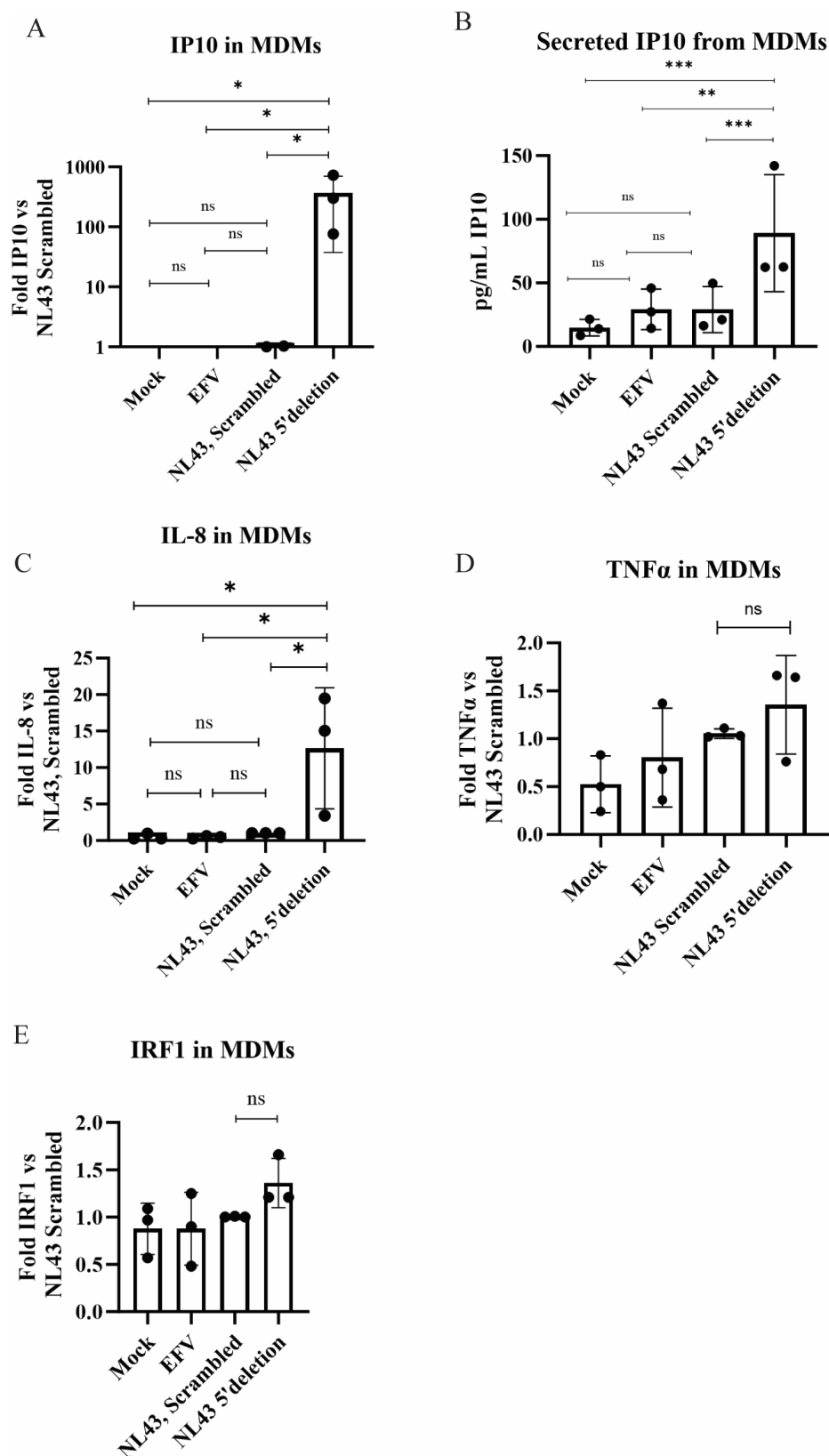


FIG 3 Engineered defective HIV-1 proviruses in MDMs induce IP-10 and IL-8. (A) RTqPCR for IP-10 in MDMs. (B) ELISA for IP-10 on supernatants from MDMs that harbor defective genomes. (C–E) RTqPCR for IL-8 (C), TNF- α (D), and IRF1 (E). Data are shown as a fold change relative to scrambled gRNA control for RT-qPCR data. The Y-axis is a log-10 scale. IL-6, IFN2 α , and IFN γ (Continued on next page)

Fig 3 (Continued)

transcripts were also assessed but were below the level of detection. Each data point represents an individual donor for a total of three replicates. Each condition was performed three times per donor. Error bars represent standard error, and paired Student's *t* tests **P* < 0.05.

included cells treated with scrambled guides and cells not treated with CRISPR or infected with HIV-1. Compared to wild-type THP1s, cells lacking MDA5 or MAVS were compromised in their ability to induce IP-10 transcription in response to defective HIV-1 proviruses (Fig. 5A and B). However, knocking down cGAS or RIG-I had no impact on the induction of IP-10 in response to defective provirus transcription (Fig. 5B). Overall, infection was not affected by CRISPR-Cas9 modification, as shown by a qPCR quantifying copies of *env* (Fig. 5C and D). These data support the hypothesis that intragenic-derived RNAs are sensed by MDA5, which signals through MAVS to trigger a type I IFN response. Our results are consistent with those of previous studies showing that MDA5 is necessary for detection of HIV-1 RNA-mediated induction of IFNs (30, 31, 44).

DISCUSSION

PWH on suppressive ART experience a wide range of comorbidities posited to be caused by chronic low-grade inflammation (17, 20, 36, 45). Previously, our group demonstrated that *env* had an intragenic *cis*-element that functioned as a transcriptional promoter and could facilitate production of noncanonical HIV-1 transcripts (38). To explore whether these RNAs had a functional consequence in infected cells, we utilized CRISPR-Cas9 to delete the 5' LTR of HIV-1 proviruses. Cells harboring these defective HIV-1 proviruses triggered increased expression of IP-10 and IL-8. These findings were observed in various cell types, including macrophages, which have been implicated in several end-organ chronic diseases associated with HIV-1 infection and aging (18, 19). Knocking down MDA5 or MAVS abrogated these responses, indicating that sensing of UTR-deficient RNAs is mediated by the MDA5-MAVS signaling cascade, a pathway implicated in sensing partially spliced HIV-1 transcripts. Consistent with a role in perpetuating HIV-1-associated comorbidities, we observed a correlation between noncanonical UTR-deficient RNAs and elevated levels of IP-10, TNF- α , and IL-8 in samples from PWH receiving ART.

Several groups have suggested that innate immune sensing of viral RNA, particularly unspliced HIV-1 RNA, mediates chronic inflammation. Sensing of exported HIV-1 unspliced RNA in DCs and MDMs by MDA5 and MAVS drives production of proinflammatory interferon-stimulated genes, including IP-10, ISG15, and MX1 (10, 30, 31, 37, 46). Total HIV-1 RNA has been shown to correlate significantly with levels of proinflammatory cytokines in serum from PWH in some studies, although others show no correlations with total HIV-1 RNAs (47–50). While total HIV-1 RNAs did not reach significant correlations in this study, there was a trend of positive correlation between total HIV RNA and TNF- α . However, prior studies have not included UTR-deficient RNAs as a key predictor of cytokine levels, instead using the UTR sequence to identify HIV-1 RNAs or a single target site within the provirus (48, 49, 51). Furthermore, the structure of the 5' leader region of viral RNA plays an important role in triggering the MDA5 response (44). Unspliced RNAs and active HIV-1 protease also trigger inflammasomes, interleukin-1 beta (IL-1 β) production, and pyroptosis (28, 29, 44, 52, 53). We propose that HIV-1 mRNAs, through multiple mechanisms, activate and dysregulate multiple intracellular innate immune responses in myeloid cells such as DCs and macrophages to synergistically drive inflammation.

Although our data support that HIV-1 noncanonical RNA is inducing an interferon response, it is possible that these mRNAs are translated and that these proteins contribute to HIV-1 pathogenesis. The mRNAs generated from the intragenic promoter with *env* contain an intact open reading frame (ORF) and are 5' capped and polyadenylated, therefore having features of mRNAs that are translated. We previously described the detection of proteins associated with these *env* transcripts by immunoblotting using

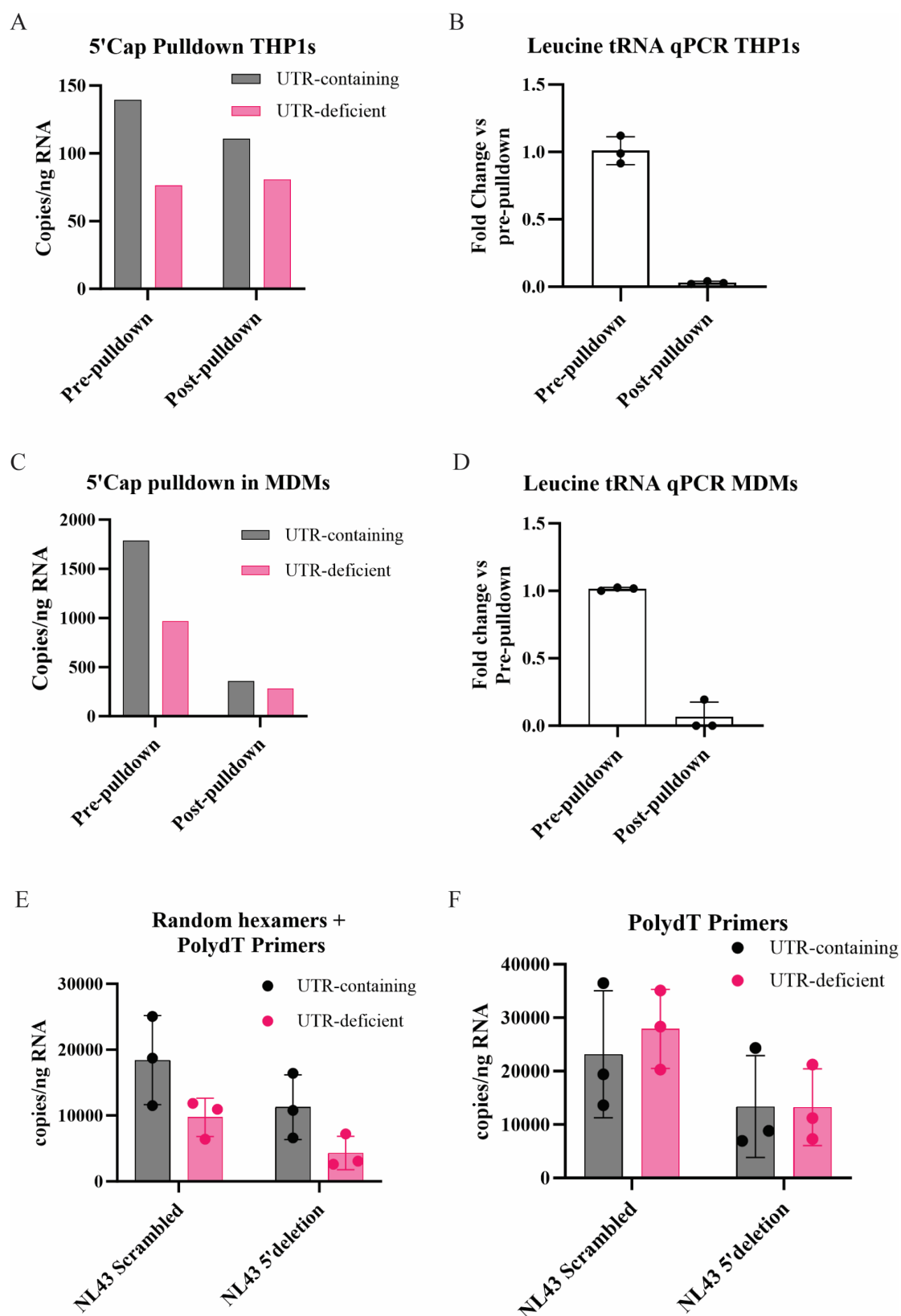


FIG 4 Defective HIV-1 RNAs are capped and poly-A tailed. (A) RNA was isolated from PMA-differentiated THP1 cells harboring CRISPR-modified defective provirus. A portion of this RNA was bound to eIF4e-GFP-coated beads, which were used to capture 5' capped mRNA. Levels of UTR-containing and UTR-deficient transcripts were determined using multiplex RTddPCR. Results are shown for RNAs pre- and post-pulldown. (B) RTqPCR for leucine-tRNA, which does not contain (Continued on next page)

Fig 4 (Continued)

a 5' cap, in PMA-differentiated THP1s pre- and post-pulldown for 5' capped RNAs. (C) Levels of 5' capped RNA from MDMs harboring defective provirus, as described for FIG 4A. (D) RTqPCR for leucine-tRNA, which does not contain a 5' cap, in MDMs. (E–F) RNA was isolated from MDMs infected with NL4-3 and transfected with scrambled or 5' deleting CRISPR RNPs. cDNA was prepared using a mixture of random hexamers and polydT primers (E) or polydT primers alone (F) and then analyzed with multiplexed RTddPCR. Each data point represents an independent experiment, with a total of three replicates per cell type (THP1 or MDM).

anti-sera from PWH (38). Several studies have shown that defective proviruses have the capacity to generate peptides, which are presented as antigens, eliciting CD8+ T cell responses (14, 26, 27, 54, 55). Several ORFs generated from alternative splicing and specific defects give rise to full-length HIV-1 proteins, truncated HIV proteins, and cryptic HIV-1 peptides, and these could shape the innate immune response and the anti-HIV-1 T-cell responses (14, 56, 57). For example, Gag and Nef proteins have been detected in PWH receiving suppressive ART and are suggested to trigger innate immune responses contributing to a failure of CD4+ T cells in PWH who are immunological nonresponders (14, 58). Furthermore, Pollock et al. suggested that class I presentation of peptides from defective proviruses elicits CTL responses, either diverting or perpetuating CD8+ responses in PWH (54). We speculate that a subset of RNAs we observed, in addition to triggering a response through MAVS, are translated into proteins that directly or indirectly skew innate and T cell responses.

Based on our findings, we propose a model for defective provirus-driven inflammation. An intragenic element within *env* remains transcriptionally active, even during latency and suppressive ART regimens. RNAs transcribed from this element are modified with a 5' cap. MDA5 senses these RNAs, triggering an interferon response through MAVS, leading to increased production of cytokines, including IP-10 and IL-8. In addition, we hypothesize that these RNAs are translated and further skew innate and adaptive responses. The continuous expression from this region in PWH drives chronic inflammation. Overall, we propose that these HIV-1 RNAs contribute to an inflammatory micro-environment that directly perpetuates HIV-1-associated comorbidities. These studies indicate that persistent defective HIV-1 proviruses are critical in long-term chronic HIV-1 pathogenesis, and their role in HIV-1-associated comorbidities needs to be considered as ongoing treatments and cure strategies are explored (Fig. 6).

MATERIALS AND METHODS

Virus preparation

HIV-1 viruses were packaged and pseudotyped with VSVg envelope by transfecting HEK-293T cells as previously described (38). Viruses were concentrated through a 20% sucrose gradient. Virus titers were obtained as previously described by infecting a CEM-R5-GFP reporter line, which produces GFP in response to Tat production, and measuring infection percentage by flow cytometry to determine the multiplicity of infection (MOI) (38). All infections were performed with an MOI of 1.0, except for Jurkat cells, which were infected with an MOI of 0.2.

Infection with HIV-1

Virus was added to Jurkat cells, and cultures were spinoculated at $1,200 \times g$ for 90 min. Cells were washed with PBS and resuspended in fresh media following spin. Samples were harvested for supernatants, RNA, and DNA at the indicated time points.

Differentiated THP1 cell lines and MDMs were incubated with virus overnight. Cells were washed with PBS and provided with fresh culture media. CRISPR-Cas9 RNPs were added 24 h post-infection. THP1 samples were harvested 48 h post-infection. MDM samples were harvested 72 h post-infection.

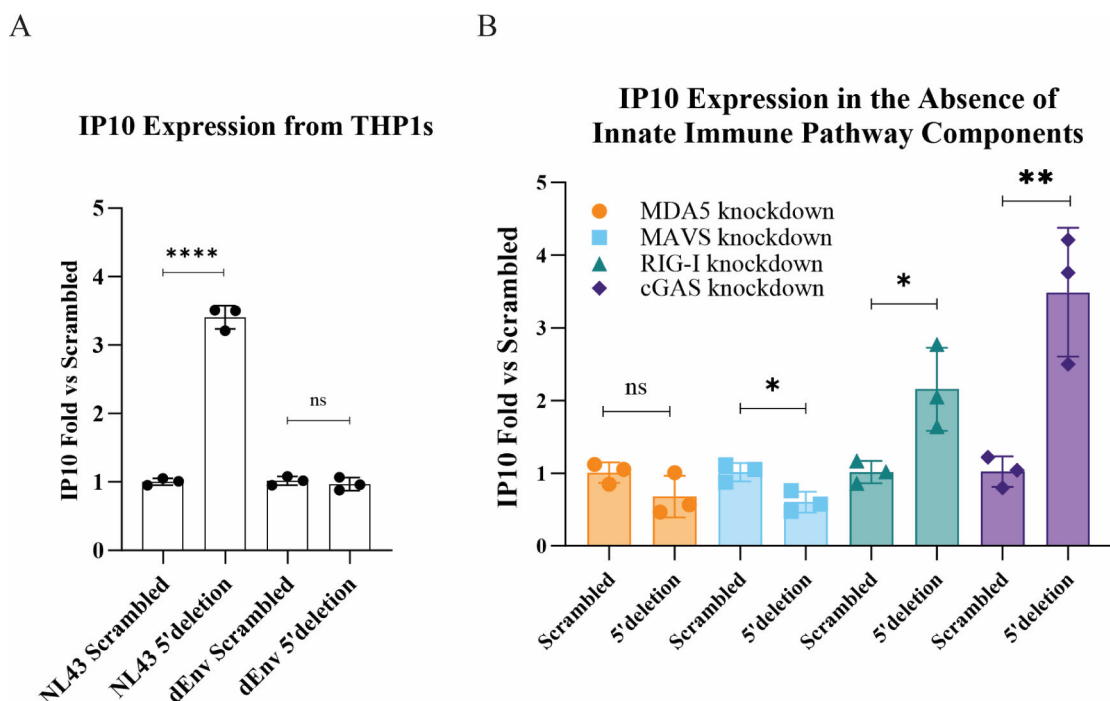


FIG 5 MAVS and MDA5 are necessary for induction of IP-10 by defective HIV-1 proviruses. (A) IP-10 RTqPCR using RNA from wild-type PMA-differentiated THP1s. THP1s were infected with either VSVg-pseudotyped NL4-3 or VSVg-pseudotyped NL4-3-dEnv-GFP and transfected with either scrambled or 5' deleting CRISPR-Cas9 RNPs. (B) MDA5 knockdowns, MAVS knockdowns, cGAS knockdowns, and RIG-I knockdowns infected with NL4-3 and transfected with either scrambled or 5' deleting CRISPR-Cas9 RNPs. Error bars represent standard error, and ANOVA and unpaired Student's *t* tests following Tukey's correction. ****P* < 0.001. There are three replicates per condition in this figure.

Isolation of PBMCs

Blood packs were obtained from Rhode Island Blood Center. Blood was diluted in PBS and separated over a Ficoll-Hypaque gradient (Stem Cell Technologies Catalog No. 7801). PBMC buffy coats were removed from the interface of plasma and the density gradient and washed with PBS. Cells were treated with red blood cell lysis buffer (155 mM NH_4Cl , 12 mM NaHCO_3 , and 0.1 mM EDTA), washed with PBS, and resuspended in selection media (PBS + 2% FBS + 2 mM EDTA). PBMCs were stored in liquid nitrogen for long-term storage. For the purification of CD14⁺ monocytes, a Stem Cell CD14⁺ human selection kit was used (Stem Cell Technologies Catalog No. 17858). Monocytes were plated in RPMI with 1% Pen/Strep (Thermo Fisher catalog no. 15140122), 1% L-glutamine (Thermo Fisher catalog no. 25030081), 5% heat-inactivated FBS (Phoenix Scientific catalog no. PS-100), 10% human AB serum (Sigma Aldrich catalog no. H4522), and 50 ng/mL M-CSF (Biolegend catalog no. 574804) for 6–7 days to differentiate into MDMs. MDMs were maintained on complete RPMI (10% FBS, 1% Pen/Strep, and 1% L-glutamine).

Cell lines

THP1 (ARP-9942) cell lines were maintained in RPMI with 1% penicillin/streptomycin (Invitrogen), 1% L-glutamine, and 10% FBS. Polyclonal THP1-cell lines constitutively knocked down for MAVS or MDA5 were engineered as previously described (37). Prior to infection, THP1 cell lines were differentiated overnight using 100 nM PMA before reseeding into 12-well plates for infection.

A polyclonal Jurkat cell line stably expressing Cas9 was generated by infecting Jurkat cells (ATCC TIB-152) with lentivirus containing the pLX_311-Cas9 vector (Addgene 96924). For lentiviral packaging, HEK293T cells (ATCC CRL-11268) were grown in DMEM +10% FBS +2 mM GlutaMAX Supplement and transfected with a DNA mixture

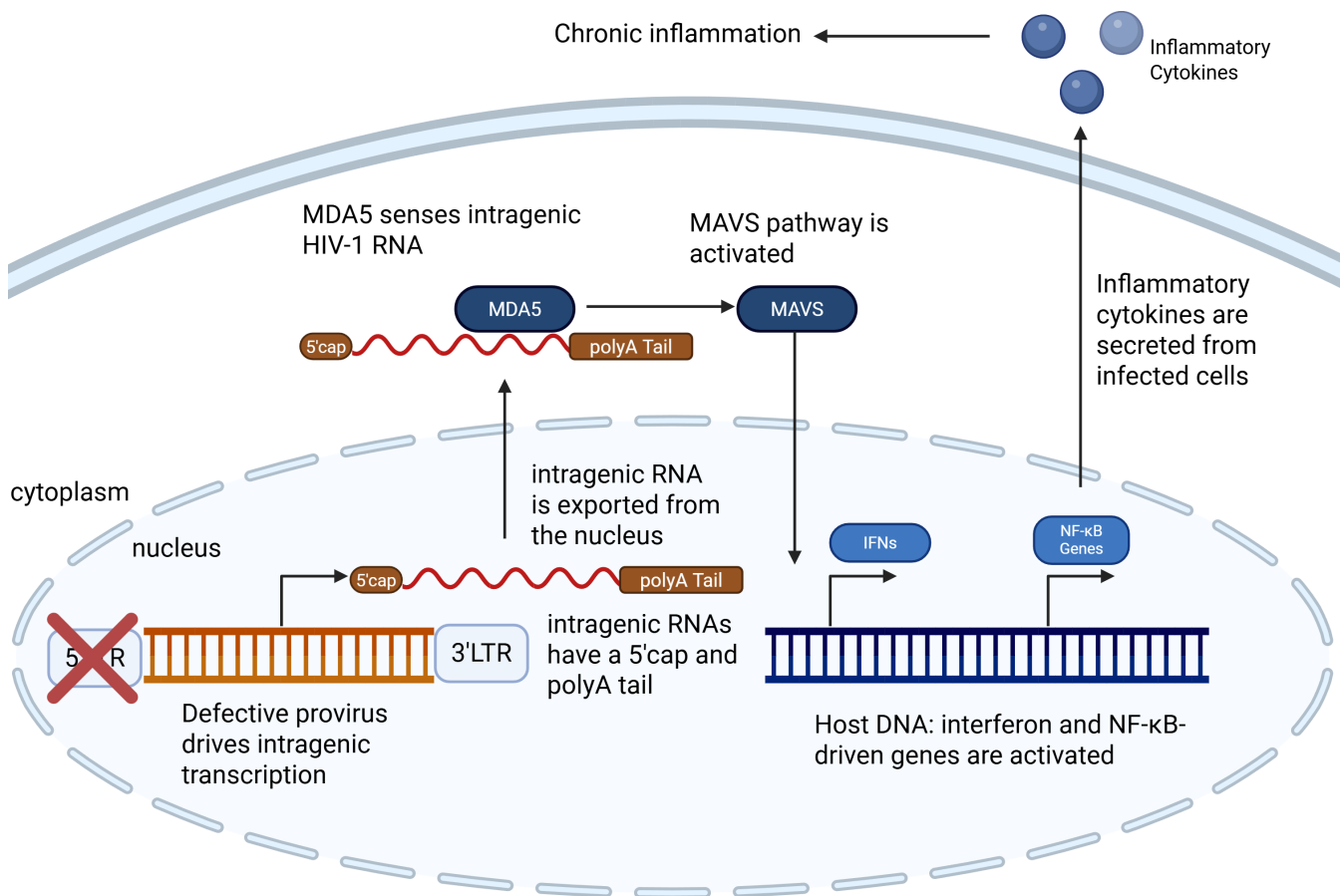


FIG 6 Proposed model for defective provirus-driven inflammation.

containing the pLX_311-Cas9 vector, the packaging plasmid psPAX2 (Addgene 12260), and envelope plasmid VSV.G (Addgene 14888) using polyethylenimine. The resulting lentivirus was concentrated using the Lenti-X Concentrator reagent (Takara 631232) according to the manufacturer's protocol. Jurkat cells were transduced using 1:5, 1:10, 1:50, 1:100, and 1:500 dilutions of Cas9 lentivirus to RPMI 1640 + FBS + GlutaMAX containing 10 μ g/mL polybrene and incubated for 48 h at 37°C, 5% CO₂. Cells were expanded for several days before selecting for the pLX_311-Cas9 vector in RPMI 1640 + FBS + GlutaMAX containing 10 μ g/mL blasticidin. The medium was changed every 3 days until cells in the non-transduced control were 0% viable, proceeding with the cell population with the highest viability. All cells were cultured at 37°C, 5% CO₂.

HEK-293-ISRE-Luc bioassay for interferons

HEK-293 cells were engineered with a lentivirus construct that contains an ISRE upstream of a luciferase gene, as described in reference 40. Supernatants are incubated with these cells for 24 h in a white-walled 96-well plate. The media/supernatant was aspirated from these plates, and 30 μ L of BrightGlo reagent (Promega Catalog No. E2610) was added to lyse the cells and measure luciferase activity. A standard curve was generated by adding a serial dilution of recombinant interferon alpha to the reporter line.

5' cap pulldown assay

5'7meG capped mRNA was isolated using a modification of the methodology from Blower et al. (59). Then, 500 ng of RNA samples was denatured by heating to 70°C for 10 min and then placing on ice. Buffer A (250 μ L) (10 mM potassium phosphate

buffer pH 8.0, 100 mM KCl, 2 mM EDTA, 5% glycerol, 0.005% Triton X-100, and 1.3% poly(vinyl) alcohol 98%–99% hydrolyzed), 1 μ L of RNase inhibitor, and 2 μ g of GFP-tagged high-affinity 5' cap-binding eIF4E (K119A) were added. Following incubation on ice for 30 min, 12.5 μ L of ChromoTek GFP-Trap Magnetic Particles M-270 was added. Following another 30-min incubation on ice, beads were washed five times with 1 mL of ice-cold Buffer A using a magnet to enrich for capped mRNAs. The resulting pellet was resuspended in TRIzol, and RNA was purified with TRIzol extraction. cDNA was prepared, and RT-ddPCR was used to quantify HIV-associated RNA. Additionally, RT-qPCR assays using Leucine-tRNA and RPL13a primers for nonspecific and specific controls were run to verify the specificity of binding of capped RNAs.

Preparation of cells for nucleic acid isolation

Following infection with HIV-1, cells were treated with 1 U/mL DNase and 100 μ M MgCl₂ for at least 30 min prior to harvesting. Adherent cells were pelleted at 500 $\times g$ for 5 min. The supernatant was removed from adherent cells and centrifuged at 500 $\times g$ for 5 min to pellet cell debris. Supernatants were then transferred to fresh tubes and stored at -20°C . RNA and DNA were isolated using the Qiagen AllPrep DNA/RNA Kit (Catalog Number 80204) or the Zymo Quick-DNA/RNA Miniprep kit (Zymo, Catalog number D7001) following the manufacturer's protocols. For some experiments, RNA was isolated using the Maxwell RSC simplyRNA Cells kit (Promega, catalog number: AS1390), and DNA was isolated using the Maxwell RSC Genomic DNA kit (Promega, catalog number: AS1880) according to the manufacturer's protocol.

CRISPR-Cas9 modification of integrated HIV

Jurkat-Cas9 cells were nucleofected using the Amaxa Lonza Nucleofector II with plasmids that expressed CRISPR gRNAs in a BW1721 backbone. These plasmids were generated by digesting BW1721 with BbsI, which cuts two sites between a U6 promoter and the gRNA scaffold sequence. Primers were designed with the target sequence for the gRNA and overhangs complementary to the BbsI cut sites (Tables S1 and S2). These primers were incubated with T4 PNK in T4 ligase buffer using T4 PNK at 37°C for 1 hour. Four microliters of 0.5 M NaCl was added to the solution, and the mixture was heated to 95°C for 5 min. The temperature was decreased by 5°C every 2 min until reaching room temperature to mediate primer annealing. SDS was used to denature the enzyme, and then PCR cleanup was performed to purify the annealed primers using the Monarch Spin PCR and DNA Cleanup Kit (New England Biolabs Catalog No. T1130L). This product and the BW1721 backbone were digested separately with BbsI at 37°C for 2 h and then purified with the PCR cleanup kit (New England Biolabs Catalog No. T1130L). The annealed primer insert and digested plasmid backbone were ligated overnight at room temperature using T4 DNA Ligase. Plasmids were validated by Sanger sequencing using a U6 promoter-specific primer to initiate sequencing. Plasmids were transformed into *E. coli* and prepared from large cultures of the transformed bacteria. Plasmids were nucleofected into Jurkat-Cas9 cells as per the manufacturer's protocols.

For THP-1 and MDMs, approximately one million cells were plated per well of a 12-well plate. Cas9, gRNAs, and Cas9 Plus Enhancer were mixed and delivered to cells as an RNP by CRISPRMAX lipofectamine (Thermo Fisher Catalog No. CMAX00003). Full-length gRNA sequences are shown in Table S2. Table S3 shows ratios of Cas9 and gRNAs.

Modification of HIV proviruses by CRISPR was confirmed by PCR using primers that flank the CRISPR-Cas9 cleavage site. Genomic DNA (10–500 ng) and 1.25 μ L of each primer were added to 25 μ L of Q5 2X master mix (New England Biolabs, catalog number M0492S) and the total volume adjusted to 50 μ L. Samples were amplified for 30–45 cycles with an annealing temperature of 61°C . PCR-amplified DNA was loaded onto a 1% agarose gel and run at 80 volts to visualize PCR products. PCR amplicons were also

sequenced. Premium PCR sequencing was performed by Plasmidsaurus using Oxford Nanopore Technology with custom analysis and annotation.

Sample analysis

Multiplexed RTddPCR assay for the quantification of HIV-1 expression and splice variants was performed as previously described (60). The assay is outlined in Fig. 1A.

RTqPCR was performed using SYBR Green Master Mix (AB Clonal Catalog No. RK21203).

Primers used for the PCRs are shown in Table S1.

ELISA measurement of cohort serum cytokines

ELISA data for donor serum cytokine were previously published data by Pihl and Smith-Mahoney et al. (39).

ELISA measurement of IP-10 from *in vitro* samples

The DuoSet Solid Phase Sandwich ELISA kit for CXCL10/IP-10 (R&D Systems, Catalog no. DY266) was used to quantify levels of IP-10 protein in the supernatant from *in vitro* experiments as per the manufacturer's protocols.

Statistical analysis

All statistics were calculated using GraphPad Prism software. PCR and ELISA data were processed using paired Student's *t* tests or ANOVA followed by Tukey's correction and paired Student's *t* tests. Analysis for clinical data correlations was run using Pearson correlations and multiple linear regression.

Cytokine values and RNA values that were below the limit of detection were set to the lowest measurable value for each assay. The logarithm of concentrations of cytokines in serum (pg/mL) and copies/ μ L/ng of HIV-1 cell-associated RNA were taken for analysis. These factors were tested for normality using D'Agostino and Pearson test, Anderson-Darling test, Shapiro-Wilk test, and Kolmogorov-Smirnov test (Table S4). The cytokines and RNA copies were normally distributed, although UTR-deficient RNAs, IL-12, and age at time of enrollment were not normally distributed (Table S5). Univariate analysis was run using a Pearson correlation test. Multiple linear regression models were run for each cytokine to determine if each subset of HIV-1 cell-associated RNA and demographic factors were good predictors.

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Jonathan M. Kilroy, Conceptualization, Formal analysis, Investigation, Methodology | Aparna A. Deokar, Formal analysis, Investigation, Methodology | Samantha Patalano, Investigation, Methodology | Juan I. Fuxman Bass, Methodology, Resources, Supervision | Manish Sagar, Formal analysis, Methodology, Supervision, Validation | Suryaram Gummuluru, Methodology, Resources, Supervision | Andrew J. Henderson, Conceptualization, Formal analysis, Funding acquisition, Methodology, Resources, Supervision, Writing – original draft, Writing – review and editing

DATA AVAILABILITY

The data underlying this study are available in the article and its supplemental material. The data are also available from the corresponding author upon request.

ADDITIONAL FILES

The following material is available [online](#).

Supplemental Material

Fig. S1 (JVI00552-26-S0001.tif). Confirmation of knockdowns by PCR.

Fig. S2 (JVI00552-26-S0002.tif). Confirmation of modified HIV-1 proviruses by sequencing.

Fig. S3 (JVI00552-26-S0003.tif). CRISPR-Cas9 RNP treatment did not impact HIV-1 infection.

Fig. S4 (JVI00552-26-S0004.tif). Targeting *hprt* in MDMs does not induce interferons.

Fig. S5 (JVI00552-26-S0005.tif). Defective HIV-1 RNAs are capped and poly-A tailed.

Fig. S6 (JVI00552-26-S0006.tif). 5' capped RPL13a mRNA.

Supplemental legends (JVI00552-26-S0007.pdf). Legends for Fig. S1 to S6.

Supplemental tables (JVI00552-26-S0008.pdf). Tables S1 to S7.

REFERENCES

1. National Institute of Health. 2025. A timeline of HIV and AIDS. Available from: <https://www.hiv.gov/hiv-basics/overview/history/hiv-and-aids-timeline>
2. UNAIDS. 2025. Global HIV & AIDS statistics — fact sheet Available from: <https://www.unaids.org/en/resources/fact-sheet>
3. National Institute of Health. 2025. The HIV life cycle. Available from: <http://hivinfo.nih.gov/understanding-hiv/fact-sheets/hiv-life-cycle>

4. Chun TW, Engel D, Berrey MM, Shea T, Corey L, Fauci AS. 1998. Early establishment of a pool of latently infected, resting CD4(+) T cells during primary HIV-1 infection. *Proc Natl Acad Sci USA* 95:8869–8873. <https://doi.org/10.1073/pnas.95.15.8869>
5. Liu R, Simonetti FR, Ho YC. 2020. The forces driving clonal expansion of the HIV-1 latent reservoir. *Viol J* 17:4. <https://doi.org/10.1186/s12985-019-1276-8>
6. Ho Y-C, Shan L, Hosmane NN, Wang J, Laskey SB, Rosenbloom DIS, Lai J, Blankson JN, Siliciano JD, Siliciano RF. 2013. Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. *Cell* 155:540–551. <https://doi.org/10.1016/j.cell.2013.09.020>
7. Liu R, Catalano AA, Ho YC. 2021. Measuring the size and decay dynamics of the HIV-1 latent reservoir. *Cell Rep Med* 2:100249. <https://doi.org/10.1016/j.xcrm.2021.100249>
8. Marsden MD, Zack JA. 2010. Establishment and maintenance of HIV latency: model systems and opportunities for intervention. *Future Virol* 5:97–109. <https://doi.org/10.2217/fvl.09.70>
9. Campbell JH, Hearps AC, Martin GE, Williams KC, Crowe SM. 2014. The importance of monocytes and macrophages in HIV pathogenesis, treatment, and cure. *AIDS* 28:2175–2187. <https://doi.org/10.1097/QAD.0000000000000408>
10. Akiyama H, Jalloh S, Park S, Lei M, Mostoslavsky G, Gummuluru S. 2021. Expression of HIV-1 intron-containing RNA in microglia induces inflammatory responses. *J Virol* 95:e01386–20. <https://doi.org/10.1128/JVI.01386-20>
11. Rausch JW, Parvez S, Pathak S, Capoferri AA, Kearney MF. 2024. HIV expression in infected T cell clones. *Viruses* 16:108. <https://doi.org/10.3390/v16010108>
12. Ishizaka A, Sato H, Nakamura H, Koga M, Kikuchi T, Hosoya N, Koibuchi T, Nomoto A, Kawana-Tachikawa A, Mizutani T. 2016. Short intracellular HIV-1 transcripts as biomarkers of residual immune activation in patients on antiretroviral therapy. *J Virol* 90:5665–5676. <https://doi.org/10.1128/JVI.03158-15>
13. Dornadula G, Zhang H, VanUitert B, Stern J, Livornese L Jr, Ingberman MJ, Witek J, Kedanis RJ, Natkin J, DeSimone J, Pomerantz RJ. 1999. Residual HIV-1 RNA in blood plasma of patients taking suppressive highly active antiretroviral therapy. *JAMA* 282:1627–1632. <https://doi.org/10.1001/jama.282.17.1627>
14. Imamichi H, Dewar RL, Adelsberger JW, Rehm CA, O'Doherty U, Paxinos EE, Fauci AS, Lane HC. 2016. Defective HIV-1 proviruses produce novel protein-coding RNA species in HIV-infected patients on combination antiretroviral therapy. *Proc Natl Acad Sci USA* 113:8783–8788. <https://doi.org/10.1073/pnas.1609057113>
15. Zhang L, Ramratnam B, Tenner-Racz K, He Y, Vesanan M, Lewin S, Talal A, Racz P, Perelson AS, Korber BT, Markowitz M, Ho DD. 1999. Quantifying residual HIV-1 replication in patients receiving combination antiretroviral therapy. *N Engl J Med* 340:1605–1613. <https://doi.org/10.1056/NEJM199905273402101>
16. Bender AM, Simonetti FR, Kumar MR, Fray EJ, Bruner KM, Timmons AE, Tai KY, Jenike KM, Antar AAR, Liu P-T, Ho Y-C, Raugi DN, Seydi M, Gottlieb GS, Okoye AA, Del Prete GQ, Picker LJ, Mankowski JL, Lifson JD, Siliciano JD, Laird GM, Barouch DH, Clements JE, Siliciano RF. 2019. The landscape of persistent viral genomes in ART-treated SIV, SHIV, and HIV-2 infections. *Cell Host Microbe* 26:73–85. <https://doi.org/10.1016/j.chom.2019.06.005>
17. Nasi M, De Biasi S, Gibellini L, Bianchini E, Pecorini S, Bacca V, Guaraldi G, Mussini C, Pinti M, Cossarizza A. 2017. Ageing and inflammation in patients with HIV infection. *Clin Exp Immunol* 187:44–52. <https://doi.org/10.1111/cei.12814>
18. Spudich S, González-Scarano F. 2012. HIV-1-related central nervous system disease: current issues in pathogenesis, diagnosis, and treatment. *Cold Spring Harb Perspect Med* 2:a007120. <https://doi.org/10.1101/cshperspect.a007120>
19. Al-Jabri AA. 2015. Forcing the enemy to come out and surrender: a strategy for an AIDS cure. *AIDS Res Hum Retroviruses* 31:45–46. <https://doi.org/10.1089/aid.2014.0259>
20. Grosso TM, Alcamí J, Arribas JR, Martín M, Sereti I, Tarr P, Cahn P, Clotet B, Sued O, Negro E. 2022. HIV and aging, biological mechanisms, and therapies: what do we know? *AIDS Rev* 25:79–86. <https://doi.org/10.24875/AIDSRev.21000085>
21. Pereyra F, Lo J, Triant VA, Wei J, Buzon MJ, Fitch KV, Hwang J, Campbell JH, Burdo TH, Williams KC, Abbara S, Grinspoon SK. 2012. Increased coronary atherosclerosis and immune activation in HIV-1 elite controllers. *AIDS* 26:2409–2412. <https://doi.org/10.1097/QAD.0b013e32835a9950>
22. Hunt PW, Brenchley J, Sinclair E, McCune JM, Roland M, Page-Shafer K, Hsue P, Emu B, Krone M, Lampiris H, Douek D, Martin JN, Deeks SG. 2008. Relationship between T cell activation and CD4+ T cell count in HIV-seropositive individuals with undetectable plasma HIV RNA levels in the absence of therapy. *J Infect Dis* 197:126–133. <https://doi.org/10.1086/524143>
23. Crowell TA, Gebo KA, Blankson JN, Korthuis PT, Yehia BR, Rutstein RM, Moore RD, Sharp V, Nijhawan AE, Mathews WC, Hanau LH, Corales RB, Beil R, Somboonwit C, Edelstein H, Allen SL, Berry SA, HIV Research Network. 2015. Hospitalization rates and reasons among HIV elite controllers and persons with medically controlled HIV infection. *J Infect Dis* 211:1692–1702. <https://doi.org/10.1093/infdis/jiu809>
24. Krishnan S, Wilson EMP, Sheikh V, Rupert A, Mendoza D, Yang J, Lempicki R, Migueles SA, Sereti I. 2014. Evidence for innate immune system activation in HIV type 1-infected elite controllers. *J Infect Dis* 209:931–939. <https://doi.org/10.1093/infdis/jit581>
25. Platten M, Jung N, Trapp S, Flossdorf P, Meyer-Olson D, Schulze Zur Wiesch J, Stephan C, Mauss S, Weiss V, von Bergwelt-Baildon M, Rockstroh J, Fätkenheuer G, Lehmann C. 2016. Cytokine and chemokine signature in elite versus viremic controllers infected with HIV. *AIDS Res Hum Retroviruses* 32:579–587. <https://doi.org/10.1089/AID.2015.0226>
26. Kilroy JM, Leal AA, Henderson AJ. 2024. Chronic HIV transcription, translation, and persistent inflammation. *Viruses* 16:751. <https://doi.org/10.3390/v16050751>
27. Imamichi H, Smith M, Adelsberger JW, Izumi T, Scrimieri F, Sherman BT, Rehm CA, Imamichi T, Pau A, Catalfamo M, Fauci AS, Lane HC. 2020. Defective HIV-1 proviruses produce viral proteins. *Proc Natl Acad Sci USA* 117:3704–3710. <https://doi.org/10.1073/pnas.1917876117>
28. Wang Q, Gao H, Clark KM, Mugisha CS, Davis K, Tang JP, Harlan GH, DeSelm CJ, Presti RM, Kutluay SB, Shan L. 2021. CARD8 is an inflammation sensor for HIV-1 protease activity. *Science* 371:eabe1707. <https://doi.org/10.1126/science.abe1707>
29. Jalloh S, Hughes IK, Akiyama H, Gojanovich AD, Quiñones-Molina AA, Yang M, Henderson AJ, Mostoslavsky G, Gummuluru S. 2025. Expression of intron-containing HIV-1 RNA induces NLRP1 inflammasome activation in myeloid cells. *PLoS Biol* 23:e3003320. <https://doi.org/10.1371/journal.pbio.3003320>
30. McCauley SM, Kim K, Nowosielska A, Dauphin A, Yurkovetskiy L, Diehl WE, Luban J. 2018. Intron-containing RNA from the HIV-1 provirus activates type I interferon and inflammatory cytokines. *Nat Commun* 9:5305. <https://doi.org/10.1038/s41467-018-07753-2>
31. Akiyama H, Miller CM, Ettinger CR, Belkina AC, Snyder-Cappione JE, Gummuluru S. 2018. HIV-1 intron-containing RNA expression induces innate immune activation and T cell dysfunction. *Nat Commun* 9:3450. <https://doi.org/10.1038/s41467-018-05899-7>
32. Bruner KM, Murray AJ, Pollack RA, Soliman MG, Laskey SB, Capoferri AA, Lai J, Strain MC, Lada SM, Hoh R, Ho Y-C, Richman DD, Deeks SG, Siliciano JD, Siliciano RF. 2016. Defective proviruses rapidly accumulate during acute HIV-1 infection. *Nat Med* 22:1043–1049. <https://doi.org/10.1038/nm.4156>
33. Berg RK, Melchjorsen J, Rintahaka J, Diget E, Söby S, Horan KA, Gorelick RJ, Matikainen S, Larsen CS, Ostergaard L, Paludan SR, Mogensen TH. 2012. Genomic HIV RNA induces innate immune responses through RIG-I-dependent sensing of secondary-structured RNA. *PLoS One* 7:e29291. <https://doi.org/10.1371/journal.pone.0029291>
34. Li P, Kaiser P, Lampiris HW, Kim P, Yukl SA, Havlir DV, Greene WC, Wong JK. 2016. Stimulating the RIG-I pathway to kill cells in the latent HIV reservoir following viral reactivation. *Nat Med* 22:807–811. <https://doi.org/10.1038/nm.4124>
35. Yin X, Langer S, Zhang Z, Herbert KM, Yoh S, König R, Chanda SK. 2020. Sensor sensibility-HIV-1 and the innate immune response. *Cells* 9:254. <https://doi.org/10.3390/cells9010254>
36. Akiyama H, Gummuluru S. 2020. HIV-1 persistence and chronic induction of innate immune responses in macrophages. *Viruses* 12:711. <https://doi.org/10.3390/v12070711>
37. Ramaswamy S, Akiyama H, Berrigan J, Quiñones-Molina AA, Olson AJ, Chen Y, Liang Y, Henderson AJ, Asundi A, Sagar M, Gummuluru S. 2025. The macrophage-intrinsic MDA5/IRF5 axis drives HIV-1 intron-containing RNA-induced inflammatory responses. *J Clin Invest* 135:16. <https://doi.org/10.1172/JCI187663>
38. Kuniholm J, Armstrong E, Bernabe B, Coote C, Berenson A, Patalano SD, Olson A, He X, Lin NH, Fuxman Bass JJ, Henderson AJ. 2021. Intra-genic

- providal elements support transcription of defective HIV-1 proviruses. *PLoS Pathog* 17:e1009982. <https://doi.org/10.1371/journal.ppat.1009982>
39. Pihl RMF, Smith-Mahoney EL, Olson A, Yuen RR, Asundi A, Lin N, Belkina AC, Snyder-Cappione JE. 2024. Vδ1 effector and Vδ2 γδ t-cell subsets shift in frequency and are linked to plasma inflammatory markers during antiretroviral therapy-suppressed HIV infection. *J Infect Dis* 229:1317–1327. <https://doi.org/10.1093/infdis/jiae091>
 40. Larocque L, Bliu A, Xu R, Diress A, Wang J, Lin R, He R, Girard M, Li X. 2011. Bioactivity determination of native and variant forms of therapeutic interferons. *J Biomed Biotechnol* 2011:174615. <https://doi.org/10.1155/2011/174615>
 41. Jefferies CA. 2019. Regulating IRFs in IFN driven disease. *Front Immunol* 10:325. <https://doi.org/10.3389/fimmu.2019.00325>
 42. Günthner R, Anders H-J. 2013. Interferon-regulatory factors determine macrophage phenotype polarization. *Mediators Inflamm* 2013:731023. <https://doi.org/10.1155/2013/731023>
 43. Babadei O, Strobl B, Müller M, Decker T. 2024. Transcriptional control of interferon-stimulated genes. *J Biol Chem* 300:107771. <https://doi.org/10.1016/j.jbc.2024.107771>
 44. Hughes IK, Kharytonchyk S, Ramaswamy S, Jalloh S, Hood JB, He X, Henderson AJ, Akiyama H, Telesnitsky A, Gummuluru S. 2025. 5' cap sequestration is required for sensing of unspliced HIV-1 RNA by MDA5. *bioRxiv*. <https://doi.org/10.1101/2025.08.20.671346>
 45. Schmitz CRR, Maurmann RM, Guma FPCR, Bauer ME, Barbé-Tuana FM. 2023. cGAS-STING pathway as a potential trigger of immunosenescence and inflammaging. *Front Immunol* 14:1132653. <https://doi.org/10.3389/fimmu.2023.1132653>
 46. Guney MH, Nagalekshmi K, McCauley SM, Carbone C, Aydemir O, Luban J. 2024. IFIH1 (MDA5) is required for innate immune detection of intron-containing RNA expressed from the HIV-1 provirus. *Proc Natl Acad Sci USA* 121:e2404349121. <https://doi.org/10.1073/pnas.2404349121>
 47. Olson A, Coote C, Snyder-Cappione JE, Lin N, Sagar M. 2021. HIV-1 transcription but not intact provirus levels are associated with systemic inflammation. *J Infect Dis* 223:1934–1942. <https://doi.org/10.1093/infdis/jiaa657>
 48. Fombellida-Lopez C, Aguilar Ortman D, Moutschen M, Pasternak AO, Darcis G. 2025. No associations between HIV reservoir and inflammation in long-term virally suppressed dolutegravir-based ART-treated individuals. *Front Immunol* 16:1628086. <https://doi.org/10.3389/fimmu.2025.1628086>
 49. Talla A, Azevedo JLLC, Latif MB, Enriquez AB, Sanchez GP, Pelletier AN, Bal SK, Kumari S, Schuch V, Ghneim K, et al. 2026. Innate antiviral and immune functions associated with the HIV reservoir decay after anti-PD-1 therapy. *Nat Med* 32:505–517. <https://doi.org/10.1038/s41591-025-04139-y>
 50. Zerbato JM, Khoury G, Zhao W, Gartner MJ, Pascoe RD, Rhodes A, Dantanarayana A, Gooley M, Anderson J, Bacchetti P, Deeks SG, McMahon J, Roche M, Rasmussen TA, Purcell DF, Lewin SR. 2021. Multiply spliced HIV RNA is a predictive measure of virus production *ex vivo* and *in vivo* following reversal of HIV latency. *EBioMedicine* 65:103241. <https://doi.org/10.1016/j.ebiom.2021.103241>
 51. Dufour C, Ruiz MJ, Pagliuzza A, Richard C, Shahid A, Fromentin R, Ponte R, Cattin A, Wiche Salinas TR, Salahuddin S, Sandstrom T, Schinkel SB, Costiniuk CT, Jenabian M-A, Ancuta P, Routy J-P, Cohen ÉA, Brumme ZL, Power C, Angel JB, Chomont N. 2023. Near full-length HIV sequencing in multiple tissues collected postmortem reveals shared clonal expansions across distinct reservoirs during ART. *Cell Rep* 42:113053. <https://doi.org/10.1016/j.celrep.2023.113053>
 52. Ekabe CJ, Clinton NA, Kehbila J, Franck NC. 2021. The role of inflammatory activation in early HIV infection. *J Immunol Res* 2021:1487287. <https://doi.org/10.1155/2021/1487287>
 53. Wang Q, Shan L. 2022. Inflammasomes in human immunodeficiency virus type 1 infection. *Infect Dis Immun* 2:248–252. <https://doi.org/10.1097/ID9.0000000000000070>
 54. Pollack RA, Jones RB, Perte M, Bruner KM, Martin AR, Thomas AS, Capoferri AA, Beg SA, Huang S-H, Karandish S, Hao H, Halper-Stromberg E, Yong PC, Kovacs C, Benko E, Siliciano RF, Ho Y-C. 2017. Defective HIV-1 proviruses are expressed and can be recognized by cytotoxic t lymphocytes, which shape the proviral landscape. *Cell Host Microbe* 21:494–506. <https://doi.org/10.1016/j.chom.2017.03.008>
 55. Wei H, Yu D, Geng X, He Y. 2020. Defective HIV-1 envelope gene promotes the evolution of the infectious strain through recombination *in vitro*. *BMC Infect Dis* 20:569. <https://doi.org/10.1186/s12879-020-05288-w>
 56. Lee SA, Bacchetti P, Chomont N, Fromentin R, Lewin SR, O'Doherty U, Palmer S, Richman DD, Siliciano JD, Yukl SA, Deeks SG, Burbelo PD. 2016. Anti-HIV antibody responses and the hiv reservoir size during antiretroviral therapy. *PLoS One* 11:e0160192. <https://doi.org/10.1371/journal.pone.0160192>
 57. Simonetti FR, Zhang H, Soroosh GP, Duan J, Rhodehouse K, Hill AL, Beg SA, McCormick K, Raymond HE, Nobles CL, Everett JK, Kwon KJ, White JA, Lai J, Margolick JB, Hoh R, Deeks SG, Bushman FD, Siliciano JD, Siliciano RF. 2021. Antigen-driven clonal selection shapes the persistence of HIV-1-infected CD4+ T cells *in vivo*. *J Clin Invest* 131:e145254. <https://doi.org/10.1172/JCI145254>
 58. Gasca-Capote C, Lian X, Gao C, Roseto IC, Jiménez-León MR, Gladkov G, Camacho-Sojo MI, Pérez-Gómez A, Gallego I, Lopez-Cortes LE, et al. 2024. The HIV-1 reservoir landscape in persistent elite controllers and transient elite controllers. *J Clin Invest* 134:e174215. <https://doi.org/10.1172/JCI174215>
 59. Blower MD, Jambhekar A, Schwarz DS, Toombs JA. 2013. Combining different mRNA capture methods to analyze the transcriptome: analysis of the *Xenopus laevis* transcriptome. *PLoS One* 8:e77700. <https://doi.org/10.1371/journal.pone.0077700>
 60. Yukl SA, Kaiser P, Kim P, Telwate S, Joshi SK, Vu M, Lampiris H, Wong JK. 2018. HIV latency in isolated patient CD4+ T cells may be due to blocks in HIV transcriptional elongation, completion, and splicing. *Sci Transl Med* 10:430. <https://doi.org/10.1126/scitranslmed.aap9927>