HIV-1 blocks the signaling adaptor MAVS to evade antiviral host defense after sensing of abortive HIV-1 RNA by the host helicase DDX3


The mechanisms by which human immunodeficiency virus 1 (HIV-1) avoids immune surveillance by dendritic cells (DCs), and thereby prevents protective adaptive immune responses, remain poorly understood. Here we showed that HIV-1 actively arrested antiviral immune responses by DCs, which contributed to efficient HIV-1 replication in infected individuals.

We identified the RNA helicase DDX3 as an HIV-1 sensor that bound abortive HIV-1 RNA after HIV-1 infection and induced DC maturation and type I interferon responses via the signaling adaptor MAVS. Notably, HIV-1 recognition by the C-type lectin receptor DC-SIGN activated the mitotic kinase PLK1, which suppressed signaling downstream of MAVS, thereby interfering with intrinsic host defense during HIV-1 infection. Finally, we showed that PLK1-mediated suppression of DDX3–MAVS signaling was a viral strategy that accelerated HIV-1 replication in infected individuals.
expression. HIV-1 escapes detection by cGAS because host proteins, such as the cytosolic exonuclease TREX1, which digests HIV-1 DNA, and the phosphohydrodrolase SAMHD1, which limits the generation of HIV-1 DNA, limits the amount of viral DNA in the cell. However, SAMHD1-mediated restriction of DC infection does not account for the lack of DC activation. Thus, although viral RNA, DNA and proteins are recognized by various host proteins, it remains unclear how HIV-1 avoids immune surveillance in DCs.

Here we identified the RNA helicase DDX3 as a sensor for abortive HIV-1 RNA in DCs, which induced type I interferon responses and DC maturation via the signaling adaptor protein MAVS. However, simultaneous recognition of HIV-1 by DC-SIGN suppressed these responses via Raf-1-mediated activation of host factor PLK1, which impeded signaling downstream of MAVS. The identification of a rare dual MAVS (Q198K, S409F) mutant that is resistant to PLK1 inactivation, which thereby contributed to limiting the viral RNA load, demonstrated the significance of DDX3-mediated sensing for HIV-1 replication in infected individuals.

**RESULTS**

**HIV-1 innate signaling via Raf-1 blocks type I interferon responses**

Because HIV-1 activates Raf-1 (ref. 12), we investigated whether Raf-1 is involved in the suppression of type I interferon responses after HIV-1 infection of human DCs. Monocyte-derived DCs (moDCs) did not express IFNB mRNA (which encodes IFN-β) after infection with the laboratory R5-tropic strain HIV-1BaL, however inhibition of Raf-1 activity with the small-molecule inhibitor GW5074 or silencing of Raf-1 expression by RNA interference (RNAi) induced transient expression of IFNB transcripts, which peaked at 4 h after infection (Fig. 1a,b). Treatment with GW5074 also induced transient expression of various ISGs, such as ISG15, MX2, TRIM5, TRIM22 and APOBEC3G (Fig. 1c); however, it did not affect TLR4-induced IFNB expression in moDCs (Supplementary Fig. 1). Infection with HIV-1BaL induced IFNB expression in only GW5074-treated but not untreated human primary myeloid, dermal, intestinal and vaginal DCs isolated from blood, skin, intestinal and vaginal tissues, respectively (Fig. 1d). We also observed IFNB expression after infection of GW5074-treated moDCs both with different laboratory strains (R5-tropic HIV-1 BaL or X4-tropic HIV-1BaL and HIV-1Ad) and with primary R5-tropic HIV-1 strains isolated from infected patients (Fig. 1e,f).

**HIV-1 infection triggers type I interferon responses via DDX3**

Induction of IFNB expression in GW5074-treated HIV-1BaL-infected moDCs was abrogated by treatment with the reverse-transcriptase inhibitor azidothymidine (AZT; a nucleoside analog) or the integrase inhibitor raltegravir (RAL), or by silencing of TLR8 expression using RNAi (Fig. 2a). Because the sensing of HIV-1 ssRNA by TLR8 is crucial to the initiation of HIV-1 transcription, these data suggested that type I interferon responses are triggered by HIV-1 RNA transcripts. Silencing of the expression of the cDNA sensor cGAS (encoded by MB21D1) and its downstream effector STING (encoded by TME173) by RNAi did not affect IFNB expression in GW5074-treated HIV-1BaL-infected moDCs (Fig. 2b and Supplementary Fig. 2). HIV-1BaL infection induced IFNB expression in both untreated and GW5074-treated moDCs after TREX1 silencing by RNAi, and this was abrogated after silencing the expression of either cGAS or STING, as well as by treatment with the reverse transcriptase inhibitor AZT but not the integrase inhibitor RAL (Fig. 2b and Supplementary Fig. 2). GW5074 treatment further enhanced IFNB expression in TREX1-silenced HIV-1BaL-infected moDCs (Fig. 2b and Supplementary Fig. 2). Thus, there are at least two independent HIV-1-sensing pathways in DCs, a Raf-1-sensitive pathway triggered by an RNA sensor after HIV-1 transcription and the TREX1-sensitive pathway induced by the DNA sensor cGAS after reverse transcription.

To identify the RNA sensor, we silenced the expression of the RNA helicases RIG-I (encoded by DDX58) and MDA5 (encoded by IFI17), which did not interfere with IFNB expression in GW5074-treated HIV-1BaL-infected moDCs (Supplementary Fig. 2). Silencing the expression of DDX3 (encoded by DDX3X), but not DDX1 or DDX5, by RNAi abrogated IFNB expression in GW5074-treated HIV-1BaL-infected moDCs (Fig. 2c). IFNB expression was not affected by silencing of DDX3 expression in TREX1-silenced HIV-1BaL-infected moDCs or by stimulation of moDCs with lipids specific for RIG-I–MDA5, cGAS–STING or TLR4 (Supplementary Fig. 2). Rescue of DDX3 expression by transfection of a DDX3-complexing plasmid restored IFNB expression in DDX3-silenced GW5074-treated HIV-1BaL-infected moDCs (Fig. 2d), excluding off-target silencing effects. These data strongly suggested

**Figure 1** Raf-1 activation suppresses type I IFN responses after HIV-1 infection of DCs. (a–f) Real-time PCR (RT–PCR) analyses of IFNB (a,c–f) and of ISG15, MX2, TRIM5, TRIM22 and APOBEC3G (b) mRNA in monocyte-derived (a,c–f) or primary (d) DCs after infection (as indicated (a–c) or 4 h (d–f)) with the R5-tropic laboratory strain HIV-1BaL (a–e), the R5-tropic laboratory strain HIV-1SF162 and the X4-tropic laboratory strains HIV-1BaL and HIV-1Ad (e) or with primary HIV-1 isolates from infected patients (f), in the absence or presence of Raf-1 inhibition (using GW5074 (a,c–f) or by RAF1 silencing (b)), n.d., not determined. Data are mean ± s.d. of six (a,c,e), four (b,f), two (d, myeloid, vaginal, intestinal DCs) or three (d, dermal DCs) different donors. **P < 0.01 and *P < 0.05 (Student’s t-test).
that DDX3 is the Raf-1-sensitive HIV-1 sensor in moDCs. We next examined whether DDX3, like other RNA sensors\(^\text{15}\), signals via MAVS. Silencing of MAVS expression inhibited IFNB expression in GW5074-treated HIV-1\(_{\text{Bal}}\)-infected moDCs (Fig. 2e), which was restored by transfection of a MAVS-encoding plasmid (Fig. 2f). Furthermore, MAVS co-immunoprecipitated with DDX3, and vice versa, from whole-cell extracts of both untreated and GW5074-treated HIV-1\(_{\text{Bal}}\)-infected moDCs, but not from extracts of uninfected moDCs (Fig. 2g). Confocal microscopy showed that HIV-1 infection invoked partial colocalization of DDX3 around mitochondria (Fig. 2h). Thus, DDX3 triggers type I interferon responses in moDCs via MAVS after HIV-1 infection, and these responses are blocked by a Raf-1-mediated pathway.

In contrast to infection of moDCs, HIV-1\(_{\text{Bal}}\) infection induced IFNB expression in monocyte-derived macrophages, which was further enhanced by GW5074 treatment and completely abrogated after silencing of DDX3 or MAVS expression (Fig. 2i). Thus, the DDX3–MAVS sensing mechanism also exists in macrophages. We next examined whether other retroviruses also trigger DDX3. HTLV-1, despite its ability to infect moDCs (Supplementary Fig. 1), did not induce IFNB responses in either untreated or GW5074-treated moDCs (Fig. 2j). In contrast, efficient HIV-2 infection (Supplementary Fig. 1) induced IFNB expression in GW5074-treated moDCs, which was abrogated after silencing of DDX3 or MAVS expression (Fig. 2j). These results imply that triggering of
DAX3–MAVS pathway for the induction of type I interferon responses is not a shared feature of all retroviruses, but neither is it restricted to HIV-1.

**DAX3 binding to abortive HIV-1 RNA leads to MAVS activation**

Considering its role in the translation of HIV-1 transcripts[16,17], we next investigated whether DAX3 associates with MAVS within translation initiation complexes. Translation initiation complexes from nuclease-treated whole-cell extracts were retained on m7GTP-cap-bound translation initiation complexes from moDCs 3 h after HIV-1βgal infection. The cap analog m7GpppG was used as a competitor. β-actin served as a loading control. (a) RT–PCR analyses of abortive HIV-1 RNA and tat-rev HIV-1 mRNA after RNA immunoprecipitation (IP) of DAX3, MAVS or elf4G from moDCs 3 h after HIV-1βgal infection, in the absence or presence of the Raf-1 inhibitor GW5074 (b), or after silencing (siRNA) of DAX3 or MAVS expression in moDCs (c). IgG and no antibody (Ab) indicate negative controls. (d–f) RT–PCR analyses of JFN8 mRNA in moDCs 4 h after transfection with HIV-1-derived sequences or endogenous human HBB RNA sequences, containing or not the 5′m7GTP cap (cap) and/or 3′poly(A) (poly(A)) structures as indicated, after silencing of DAX3 or MAVS expression (d,f) or after cross-linking (XL) of DC-SIGN, in the absence or presence of GW5074 (e). TAR, sequence corresponding to full-length HIV-1 tat transcript; β-globin, sequence corresponding to full-length human HBB transcript. (g) RT–PCR analyses of JFN8 mRNA in 293T cells depleted of DAX3, MAVS, RIG-I and/or MDA5 by CRISPR–Cas9 genome editing 4 h after transfection with cap-TAR RNA coupled to LyoVec or with poly(IC)–LyoVec as a control. Data are representative of three independent experiments (a) or are the mean ± s.d. of three (b,c), eight (d) or four (e,f) different donors or of four independent measurements (g). *P < 0.05 and **P < 0.01 (Student’s t-test).
MAVS expression (Fig. 3d). Activation of DC-SIGN using cross-linking antibodies abrogated IFNB expression in cap-TAR-transfected moDCs, whereas GW5074 treatment restored these responses (Fig. 3e). Notably, transfection of moDCs with tat transcripts lacking their 3′ poly(A) tail (cap-Tat) but not intact transcripts (cap-Tat-poly(A)) induced IFNB expression, which was abrogated by the silencing of DDX3 or MAVS expression (Fig. 3f). DDX3 also binds some endogenous transcripts, such as HBB (which encodes β-globin). Transfection of moDCs with HBB transcripts lacking their 3′ poly(A) tail (cap-β-globin) but not with intact HBB transcripts (cap-β-globin-poly(A)) induced IFNB expression, which was abrogated by the silencing of DDX3 or MAVS expression (Fig. 3f). These results suggested that RNAs that bind DDX3 and avoid ribosomal recruitment induce MAVS signaling, independently of the (viral) nature of the RNA.

We next generated 293T cell lines that were depleted of either DDX3 (293TΔDDX3) or MAVS (293TΔMAVS) using CRISPR–Cas9-directed genome editing (Supplementary Fig. 3). Transfection of control 293T, 293TARG-1, 293TΔMA5 and 293TARG-1-ΔMA5 cells, but not of 293TΔDDX3 or 293TΔMAVS cells, with cap-TAR RNA induced IFNB expression (Fig. 3g). The RIG-1–MΔ5 ligand poly(I:C)-LyoVec induced IFNB expression in control 293T and 293TΔDDX3 cells but did not do so in 293TARG-1, 293TΔMA5, 293TARG-1-ΔMA5 or 293TΔMAVS cells (Fig. 3g). Thus, the DDX3–MAVS sensing mechanism is not restricted to DCs and macrophages.

DDX3 activates IRF3 via TRAF3 recruitment to MAVS

RIG-I and MDA5 induce recruitment of the adaptor TRAF3 to MAVS aggregates, resulting in the subsequent activation of the IκB-kinase-related kinases TBK1 and IκKε for phosphorylation of the transcription factor IRF3 (refs. 18,23). We observed that TRAF3 silencing by RNAi abrogated IFNB expression in GW5074-treated HIV-1Δtreated moDCs (Fig. 4a). TRAF3 co-immunoprecipitated with DDX3 from whole-cell extracts of GW5074-treated but not untreated HIV-1Δ-infected moDCs, and this was abrogated by silencing of MAVS expression (Fig. 4b). These results implied that Ral-1 suppresses HIV-1-induced type I interferon responses by impeding recruitment of TRAF3 to MAVS.

Silencing the expression of IRF3 or both TBK1 and IκKε by RNAi abrogated IFNB expression in GW5074-treated HIV-1Δ-infected moDCs (Fig. 4c). FACs analyses showed that HIV-1Δ-infected GW5074-treated moDCs induced phosphorylation of TBK1 and IκKε at Ser172, a mark of activation24 and that this was abrogated by silencing of DDX3 or MAVS expression by RNAi (Fig. 4d). TBK1 and IκKε phosphorylation was only observed in approximately 10% of GW5074-treated moDCs (Fig. 4d), suggesting that these kinases were only activated in productively infected cells. MAVS phosphorylation by TBK1–IκKε recruits IRF3 to MAVS, which precedes the phosphorylation of IRF3 on Ser396 by TBK1–IκKε25. Nuclear translocation and phosphorylation of IRF3 in GW5074-treated HIV-1Δ-infected moDCs was abrogated by silencing of DDX3 or MAVS expression (Fig. 4e). Recruitment of IRF3 to DDX3, as assessed by co-immunoprecipitation from whole-cell extracts of GW5074-treated HIV-1Δ-infected moDCs, was attenuated by silencing of MAVS, TRAF3 and TBK1–IκKε expression (Fig. 4b). These results indicated that, after inhibition of Ral-1 activity, sensing of abortive HIV-1 RNA by DDX3 leads to MAVS–TRAF3– and TBK1–IκKε-dependent activation of IRF3.
**Figure 5** PLK1 activation during HIV-1 infection impedes TRAF3 recruitment to DDX3–MAVS and type I interferon responses. (a,b) RT–PCR analyses of *IFNB* mRNA in moDCs after HIV-1\_Bat infection (4 h), in the absence or presence of GW5074 (a), and after silencing (siRNA) of PLK1 (a,b). (c,d) Immunoblot (IB) analyses of DDX3, MAVS and PLK1 (c) or TRAF3 (d) after IP of DDX3 from moDCs 3 h after HIV-1\_Bat infection, in the absence or presence of GW5074 (c), and after silencing of MAVS (e) or PLK1 (d) expression. (e,f) Immunoblot (e) and flow cytometry (f) analyses of Thr210 phosphorylation of PLK1 in moDCs 3 h after HIV-1\_Bat infection or gp120 stimulation (f), in the absence or presence of GW5074 (e,f) or DC-SIGN-specific blocking antibodies (f). β-actin served as loading control (e). (g) ELISA for quantification of phosphorylation of Thr210 on Flag–PLK1 by recombinant kinases. (h,i) Kinase activity assay for MST1 activity (h) or flow cytometry analyses of Thr183 autophosphorylation of MST1 (i) in moDCs after HIV-1\_Bat infection or gp120 stimulation (1 h), in the absence or presence of GW5074 (h,i) or DC-SIGN-specific blocking antibodies (h). (j) Flow cytometry analyses of Thr210 phosphorylation of PLK1 as in (e,f), except after silencing of MST1 expression. (k) Immunoblot analyses of DDX3, MAVS, PLK1 and TRAF3 after IP of DDX3 from moDCs 3 h after HIV-1\_Bat infection after silencing of MST1 expression. (l) RT–PCR analyses of *IFNB* mRNA in moDCs after HIV-1\_Bat infection (4 h), in the absence or presence of GW5074, and after silencing of MST1 expression. Data are representative of three (c,d,k), two (e,i), six (f, HIV-1), four (f, gp120) or five (j) independent experiments or are the mean ± s.d. of six (a,l) or four (b,h) different donors or of three independent measurements (g). *P < 0.05 and **P < 0.01 (Student’s *t*-test).

**HIV-1-activated PLK1 impedes TRAF3 recruitment to MAVS**

Association of the mitotic kinase PLK1 with MAVS blocks MAVS–TRAF3 interactions. Notably, PLK1 silencing by RNAi induced transient *IFNB* expression in untreated HIV-1\_Bat-infected moDCs (Fig. 5a,b), similar to that observed in GW5074-treated moDCs (Fig. 1a). PLK1 silencing in HIV-1\_Bat-infected moDCs did not abrogate the induction of *lat–rev* mRNA expression (Supplementary Fig. 4), indicating that PLK1, unlike Raf-1, is not involved in HIV-1 transcription elongation. PLK1 co-immunoprecipitated with DDX3 from whole-cell extracts of HIV-1\_Bat-infected, but not uninfected, moDCs, and this was abrogated by MAVS silencing and GW5074 treatment (Fig. 5c). Both TRAF3 and IRF3 co-immunoprecipitated with DDX3 in PLK1-silenced HIV-1\_Bat-infected moDCs (Fig. 5d and Supplementary Fig. 5). These results indicated that Raf-1 activation induces association of PLK1 with MAVS in HIV-1-infected DCs, which prevents downstream DDX3–MAVS signaling.

PLK1 resides in an inactive state due to intramolecular interactions, which are released when PLK1 becomes phosphorylated at Thr210, enabling PLK1 to bind its substrates, such as MAVS. FACs analyses showed that both HIV-1\_Bat infection and gp120 stimulation of moDCs induced PLK1 phosphorylation at Thr210 and that this was abrogated in the presence of blocking antibodies specific for DC-SIGN or by treatment with GW5074 (Fig. 5e,f), indicating that HIV-1\_Bat induces activation of Raf-1 and subsequent phosphorylation of PLK1 (Thr210) via DC-SIGN.

Recombinant Raf-1, in contrast to recombinant protein kinase A (which was used as a control), did not phosphorylate Thr210 on Flag-tagged PLK1 in a biochemical assay (Fig. 5g), suggesting that PLK1 is not a direct target of Raf-1. Ste20-like kinases have been reported to phosphorylate PLK1 at Thr210 (ref. 28). Because the Ste20-like kinase MST1 interacts with the adaptor protein CNK1 (ref. 29), a component of the Raf-1 signalosome that is attached to DC-SIGN in resting DCs, we assessed whether PLK1 is a target of MST1. Recombinant MST1 phosphorylated Flag–PLK1 at Thr210 in a biochemical assay (Fig. 5g). We furthermore observed transient activation of MST1 in whole-cell extracts from HIV-1\_Bat-infected moDCs, which was abrogated by treatment with blocking antibodies specific for DC-SIGN or with GW5074 in a kinase activity assay (Fig. 5h). GW5074-sensitive MST1 activity after HIV-1\_Bat infection or gp120 stimulation in moDCs coincided with MST1 phosphorylation at its autophosphorylation site Thr183 (ref. 30), as assessed by FACs analyses (Fig. 5i). MST1 silencing by RNAi in HIV-1\_Bat-infected moDCs abrogated both PLK1 phosphorylation (Fig. 5j) and co-immunoprecipitation of PLK1 with DDX3 from whole-cell extracts (Fig. 5k) while inducing TRAF3 and...
Type I interferon responses suppress HIV-1 replication

We next investigated the effect of single-nucleotide polymorphisms (SNPs) in genes encoding components of the DDX3–MAVS signaling pathway within a cohort of untreated HIV-1-infected ‘men who have sex with men’ (MSM cohort). We identified three SNPs within MAVS—rs7262903, rs7269320 and rs7267297—that showed significant differences in plasma viral load. rs7262903 and rs7269320 results in amino acid substitutions within MAVS (Gln198Lys (Q198K) and Ser409Phe (S409F), respectively), whereas rs7267297 is located within the sequence that encodes the 3’ untranslated region (UTR) of the MAVS mRNA. rs7262903 and rs7269320 are 100% linked within the MSM cohort (n = 6); linkage disequilibrium in the global population is D’ = 0.956, r2 = 0.559 (HapMap release 23). Notably, plasma viral load at set point was significantly lower in HIV-1-infected individuals homozygous for the minor alleles of rs7262903 and rs7269320 (minor genotype) than in individuals homozygous for the major alleles (major genotype) (Fig. 6b), whereas no differences were detected in the plasma viral loads at set point between individuals with heterozygous and major genotypes (Fig. 6b). We observed a significant delay in the time from seroconversion to viral RNA load >105 copies/ml plasma, which was the median HIV-1 RNA load in the cohort31, in individuals with the minor genotype than in those with the major genotype (Fig. 6c). There was no significant effect on disease progression (AIDS, according to the US Centers for Disease Control definition, 1993) over the course of 180 months in individuals with the minor genotype, probably because of the low number of individuals with this genotype (6/304) within the MSM cohort (data not shown). These data indicated that the DDX3–MAVS pathway was important in controlling HIV-1 replication, not only during the acute infection stage but also in the clinical latency stage.

We next examined the effect of the MAVS(Q198K,S409F) protein, which is encoded by minor genotype alleles, on HIV-1 replication in DCs from healthy donors. We observed a lower percentage of p24+ moDCs derived from healthy individuals with the minor genotype (minor genotype moDCs) after HIV-1 measured infection than in those from individuals with the major genotype (major genotype moDCs), whereas treatment with IFNR-specific blocking antibodies enhanced infection to levels similar to those observed in major genotype moDCs (Fig. 6d), indicating that suppression of type I interferon responses was defective in HIV-1-infected DCs expressing MAVS(Q198K,S409F).

Both major and minor genotype moDCs responded similarly to RIG-I–MDA5 ligands, such as poly(I:C)-Lyovect or the measles virus, for the induction of IFNB expression (Fig. 7a), indicating that MAVS(Q198K,S409F) is functionally neutral, as reported for MAVS(Q198K)32. HIV-1 measured infection induced IFNB and ISG expression in both untreated and GW5074-treated minor genotype moDCs (Fig. 7bc), in contrast to that observed in heterozygous genotype moDCs, which induced only IFNB expression after GW5074 treatment (Fig. 7b), similar to that observed in major genotype moDCs (Fig. 1a). We also observed IFNB expression after HIV-1 measured infection of both untreated and GW5074-treated primary dermal DCs isolated from healthy minor genotype individuals (Fig. 7a). IFNB expression in HIV-1 measured-infected minor genotype moDCs was abrogated by silencing of DDX3 or MAVS expression (Fig. 7e). Transfection of a plasmid expressing wild-type MAVS in MAVS-silenced minor genotype moDCs rescued IFNB responses that were induced by HIV-1 measured infection only after GW5074 treatment (Supplementary Fig. 6), mimicking the responses in major genotype moDCs and indicating that the identified MAVS SNPs mediate the observed effects. DDX3 co-immunoprecipitated with MAVS and TRAF3, but not with PLK1, in whole-cell extracts from HIV-1 measured-infected minor genotype moDCs (Fig. 7f). Also, PLK1 silencing by RNAi did not affect IFNB expression in HIV-1 measured-infected minor genotype moDCs (Fig. 7f). FACS analyses showed that Thr210 phosphorylation on PLK1 was normally induced by HIV-1 measured infection in minor genotype moDCs (Fig. 7h). However, Thr210-phosphorylated Flag–PLK1 immunoprecipitated

IRF3 co-immunoprecipitation with DDX3 (Fig. 5k), IRF3 activation (Supplementary Fig. 5) and IFNB expression (Fig. 5i). Similar to that seen with Ralf-1 inhibition, MST1 silencing blocked tat–rev mRNA expression in HIV-1 measured-infected moDCs (Supplementary Fig. 1), suggesting that MST1 is involved in HIV-1 transcription elongation. Taken together, these data indicated that HIV-1 binding to DC-SIGN triggers Ralf-1-dependent MST1 activation, which leads to PLK1 phosphorylation and subsequent PLK1 binding to MAVS, thus blocking DDX3–MAVS signaling.

Figure 6 Type I interferon responses by DCs suppress HIV-1 replication in infected individuals. (a) Flow cytometry analyses of viral p24 expression in moDCs (percentage p24+ cells) at 3, 5 and 7 d after HIV-1 measured infection, in the absence or presence of IFNR-specific blocking antibodies, and after silencing (siRNA) of PLK1 expression. (b) HIV-1 RNA levels (log_{10} viral copies/ml plasma) at set point in untreated HIV-1-infected individuals in a human MSM cohort, differentiated based on genotype of linked MAVS SNPs rs7262903 and rs7269320 (minor, homozygous for minor alleles; Hz, heterozygous for both alleles; major, homozygous for major alleles). (c) Kaplan–Meier survival curve comparing the time required for log_{10} viral copies/ml plasma to reach >4.5 from time of seroconversion in untreated HIV-1-infected individuals in a human MSM cohort, differentiated by genotype as in b. (d) Flow cytometry analyses of viral p24 expression in moDCs, as in a, in moDCs expressing either wild-type MAVS (major genotype: homozygous for major alleles of rs7262903 and rs7269320) or MAVS(Q198K,S409F) (minor genotype). Data are representative of four (a), five (d), major or three (d), minor independent experiments (mean ± s.d. of duplicates (a,d) or of n = 304 patients (b,c)). P = 0.044, n.s., not significant (P = 0.215) (unpaired Student’s t-test) (b); P = 0.025; hazard ratio (HR) = 0.166, 95% confidence interval 0.015–0.759 (Cox regression model) (c).
MAVS from lysates of major genotype moDCs but not minor genotype moDCs (Fig. 7i). We next investigated whether either Q198K or S409F or both mutations in MAVS were required to block PLK1–MAVS interactions. HIV-1Bal infection induced IFNB expression in MAVS-silenced moDCs that were complemented with plasmids expressing MAVS(Q198K), MAVS(S409F) or MAVS(Q198K,S409F), in contrast to the control-siRNA-silenced moDCs that still expressed wild-type MAVS (Fig. 7j), indicating that both mutations are required for PLK1-mediated MAVS suppression.

Similar to infection with HIV-1Bal, infection of moDCs with vesicular stomatitis virus (VSV)-G-pseudotyped HIV-1 (HIV-1VSV-G) does not result in type I interferon responses12,13,14. During HIV-1VSV-G infection, retroviral integration and transcriptional initiation occur in a similar time frame as that observed for HIV-1Bal; however, the VSV-G envelope does not bind DC-SIGN12. Infection of minor genotype moDCs but not major genotype moDCs with HIV-1VSV-G induced IFNB expression 4 h after infection, and this was abrogated by silencing of DDX3 expression (Fig. 7k), indicating that HIV-1VSV-G induces inhibition of MAVS signaling independently of DC-SIGN. Thus, both HIV-1 and VSV-G target MAVS for suppression of IFN-β expression.

Attenuation of DDX3–MAVS signaling suppresses immune activation

We next addressed whether suppression of DDX3–MAVS signaling prevented DC activation in response to HIV-1 infection. We only observed enhanced expression of the maturation markers CD80, CD83 and CD86 in GW5074-treated but not in untreated HIV-1Bal-infected moDCs by FACS analyses, which was abrogated by treatment with IFNAR-specific blocking antibodies or by silencing of DDX3 or MAVS expression by RNAi (Fig. 8a,b). In contrast, HIV-1Bal-infected PLK1-silenced or minor genotype moDCs induced expression of these maturation markers even without GW5074 treatment, which was inhibited by treatment with IFNAR-specific blocking antibodies (Fig. 8a,b). These data strongly indicated that type I interferon responses are essential for DC maturation. Thus, HIV-1 targets DDX3–MAVS signaling not only to block antiviral responses but to block adaptive immune responses as well (Supplementary Fig. 7).

Figure 7 PLK1-mediated suppression of DDX3 signaling after HIV-1 infection is impeded by mutation of MAVS. (a) RT–PCR analyses of IFNB mRNA in moDCs with either major or minor genotype for MAVS SNPs rs7262903 and rs7269320 6 h after transfection with poly(I:C)-LyoVec, 24 h after infection with measles virus (MV). (b–e) RT–PCR analyses of IFNB (b,d,e) and of ISG15, MX2, TRIM5, TRIM22 and APOBEC3G (c) mRNA in moDCs (b,c,e) or primary dermal DCs (d) with either heterozygous (Hz) (b) or minor genotypes (b,e), after HIV-1Bal infection (4 h in d,e), in the absence or presence of GW5074, and after silencing of DDX3 or MAVS expression (e). (f) Immunoblot (IB) analyses for the indicated proteins after IP of DDX3 from minor genotype moDCs 3 h after HIV-1 infection, in the absence or presence of GW5074, –actin served as loading control. (g) RT–PCR analyses of IFNB mRNA in minor genotype moDCs, in the absence or presence of GW5074, and after silencing of PLK1 expression. (h) Flow cytometry analyses of Thr210 phosphorylation on PLK1 in minor genotype moDCs 3 h after HIV-1Bal infection, in the absence or presence of GW5074 or DC-SIGN-specific blocking antibodies. (i) ELISA of association between Thr210-phosphorylated Flag–PLK1 and MAVS in lysates from either major or minor genotype moDCs. (j) RT–PCR analyses of IFNB mRNA in MAVS-silenced moDCs 4 h after HIV-1Bal infection, in the absence or presence of GW5074, after complementation of MAVS expression by transfection of plasmids expressing RNAI-resistant cDNAs encoding single (QF, Q198K,S409F; KS, Q198K,S409G) or double (KF, Q198K,S409F) amino acid substitutions in MAVS. (k) RT–PCR analyses of IFNB mRNA in moDCs with either the major or minor genotype for MAVS SNPs rs7262903 and rs7269320 4 h after infection with HIV-1VSV-G, after silencing of DDX3 expression. Data are representative of three independent experiments (f–h) or are the mean ± s.d. of two (d, minor genotype; k, three (a,b, minor genotype; c,e,g,i) or four (b, Hz genotype; j) different donors. *P < 0.05 and **P < 0.01 (unpaired Student’s t-test (i) or Student’s t-test (j)).
**DISCUSSION**

HIV-1 avoids immune surveillance required for the mounting of protective responses. Here we identified the RNA helicase DDX3 as an intracellular sensor that coupled recognition of abortive HIV-1 RNA to the induction of antiviral responses. DDX3 is ideally suited as a HIV-1 RNA sensor, as it is also essential for HIV-1 replication by facilitating nuclear export and translation of HIV-1 transcripts. All HIV-1 transcripts—spliced, unspliced, abortive or full-length—share the same highly structured ~58 nt of the 5' UTR and interact with DDX3. DDX3 bound to abortive HIV-1 RNAs did not assemble in translation complexes, as these abortive RNAs lack a poly(A) tail, which prevents PABP binding. Instead, the DDX3-abortive RNA complexes associated with MAVS, which led to antiviral responses. The roles of DDX3 in HIV-1 translation and antiviral defense are thus mutually exclusive, which might prevent triggering of antiviral immune responses to endogenous transcripts that also interact with DDX3 (ref. 16).

Abortive HIV-1 RNAs are generated early after integration and, therefore, DDX3-mediated type I interferon responses were induced very rapidly, within 4 h after infection, as opposed to antiviral responses to VSV-G-pseudotyped HIV-1, which are detected 22 h after infection of Vpx-treated DCs. Abortive HIV-1 RNAs are also found in high amounts in latently infected cells, and it will be interesting to examine whether they can induce type I interferon responses via DDX3.

HIV-1 infection blocked DDX3 signaling at the level of TRAF3 recruitment to MAVS via DC-SIGN-dependent activation of PLK1. Our data underline the importance of host innate receptors for HIV-1 in suppressing antiviral host defenses and show the parallel existence of various HIV-1-sensing mechanisms in DCs that are restricted in different ways. While cGAS signaling after sensing of cDNA is blocked by the presence of the host exonuclease TREX1 (ref. 20), signaling by DDX3 after sensing of abortive transcription products was blocked by simultaneous activation of the host factor PLK1 via DC-SIGN after HIV-1 infection. Our data also showed that viral blocking of type I interferon responses by interference at the level of MAVS was not limited to DC-SIGN signaling, as VSV-G-pseudotyped HIV-1 also inhibited MAVS signaling, even though the VSV-G envelope does not engage DC-SIGN. MAVS targeting or PLK1 activation might be a versatile tool for different viruses to evade immune responses. PLK1 activation can be achieved by a variety of kinases, mostly by members of the broad family of Ste20-related serine–threonine kinases, and PLK1 activation by HIV-1 can be achieved by tumor necrosis factor, which is induced after infection with HIV-1 but not with HIV-1 (ref. 12). These observations also signify that the interpretation of experiments using VSV-G-pseudotyped viruses or particles should take into account the distinct effects of the VSV-G envelope.

PLK1 has a critical role in cell division; however, its role in non-dividing, differentiated cells is poorly defined. Our data identified PLK1 as an inhibitor of antiviral defense against HIV-1 in DCs. Although PLK1 association with MAVS blocked recruitment of TRAF3 to MAVS, the exact underlying mechanism remains to be elucidated. PLK1 could block aggregation of MAVS, which is a prerequisite for downstream signaling by MAVS for IRF3 activation. Our data suggest that PLK1 inhibitors could constitute a novel class of antiretroviral drugs that enhances endogenous antiviral immunity in individuals potentially infected with HIV-1.

The importance of early type I interferon responses in HIV-1 infection was substantiated by the identification of two linked SNPs within the MAVS gene that encode a double mutant, MAVS(Q198K,S409F). Our data showed that this double mutant was insensitive to PLK1 suppression and could thereby orchestrate
an effective antiviral defense after HIV-1 infection via the induction of DC maturation and a type I interferon response. Notably, we observed that markedly prolonged suppression of plasma viral load in individuals infected with HIV-1 in a human MSM HIV-1 infection cohort was associated with homozygosity for the rare MAVS allele that encodes this double mutant. The exact consequences of type I interferon responses during HIV-1 infection are a topic of debate. Inflammation during infection is predictive of non-AIDS morbidity and death, as well as high set point plasma virus load. Type I interferon responses are especially important in limiting viral spread; however, during HIV-1 infection, they might also result in more T cell activation and therefore more HIV-1 target cells. Inhibition of type I interferon responses in nonhuman primates early during SIV infection decreases the expression of genes involved in antiviral responses, increases the SIV reservoir size and results in faster T cell depletion with progression to AIDS, whereas continuous exposure to type I interferon leads to desensitization and increases disease progression. Our data in untreated patients with HIV-1 who express MAVS(Q198K,S409F) corroborated that early antiviral responses during infection are beneficial in host control of viral replication also during the clinical latency stage. Localized vascular application of type I interferon shortly before HIV-1 exposure decreases viral replication in humanized mice. Our observation that inhibition of the DDX3–MAVS blockade in primary human vaginal DCs after HIV-1 infection restored type I interferon responses would suggest that topical therapeutic targeting could be beneficial during the process of sexual transmission of the virus. Overall, our study underscores the importance of antiviral type I interferon responses in acute retroviral exposure, during which time DCs are a prominent target for HIV-1, which is also required to limit viral replication at the clinical latency stage, and reveals the identified pathways as important novel targets for early therapeutic intervention to boost endogenous antiviral immunity in acute exposure or even as a prophylactic measure.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS


COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

**Dendritic cell and macrophage isolation.** Monocyte-derived DCs were generated from the blood of healthy volunteer donors (Sanquin) as described44. Monocyte-derived macrophages were generated from isolated monocytes by culturing in RPMI medium (Gibco) with 10% FCS and 50 ng/ml macrophage colony-stimulating factor (M-CSF; Immunotools) for 7 d, while 500 U/ml IL-4 (Invitrogen) was added for the last 48 h. Primary DCs were isolated from the blood of healthy volunteer donors, skin and vaginal tissues were obtained from healthy individuals undergoing reconstructive plastic procedure or vaginal prolapso surgery, respectively, and healthy bowel tissues were obtained from patients with cancer or those with colitis ulcerosa undergoing colectomy. Tissue harvesting procedures were approved by the AMC Medical Ethics Committee. Split-skin grafts 0.3 mm in thickness were obtained using a dermatome (Zimmer) and digested for 45 min at 37 °C with 2 µg/ml dispase II (Roche Diagnostics) to separate the dermis from the epidermis. Dermis and vaginal tissues were further treated with 5 mg/ml collagenase D and 200 U/ml DNase I (both from Roche Diagnostics) for 90 min at 37 °C after mechanical fragmentation with forceps and scissors. Mucosa from bowel tissues was separated from underlying tissues using scissors and digested with 1 mg/ml collagenase D, 50 µg/ml DNase I (both Roche diagnostics) and 1 mg/ml soybean trypsin inhibitor (Invitrogen) for 1 h at 37 °C; this was followed by mechanical dissection using the GentleMACS Dissociator (Miltenyi Biotec). Digested tissues were then passed over a 100-µm cell strainer (Greiner) to obtain single-cell suspensions. DC-SIGN+ cells were then isolated using a CD209 MicroBead kit (Miltenyi Biotec). Blood and tissue samples were routinely screened for HTLV-1 or HIV-1 (sequences corresponding to either abortive HIV-1 RNA, full-length HIV-1 gp120 (NIH AIDS Research and Reference Reagent Program), or 10^9 1293T cells were transfected with 25 nM siRNA using transfection reagent (Invitrogen) for 2 h: 1 as described. Preincubation with inhibitors or blocking antibodies was done otherwise stated.

**Viruses and infection.** HIV-1, HIV-2 and HTLV-1 were obtained from infected cell lines and were propagated in 293T cells. HIV-1 R9 and HIV-1 BaL were obtained from infected cell lines and were propagated in 293T cells. Cells were transfected with 2 µg CRISPR-Cas9 expressing knockout plasmids (control, sc-418922; DX3x, sc-419975; MAVS, sc-400769; RIG-I, sc-432915; MDA5, sc-401962; all from Dharmacon) and used for experiments 72 h later. Cells were then passed over a 100-µm cell strainer (Greiner) to obtain single-cell suspensions. DCs were transfected with 25 nM siRNA using transfection reagent (Invitrogen) for 2 h: 1 as described. Preincubation with inhibitors or blocking antibodies was done otherwise stated.

**Immune responses.** IFNB and ISG mRNA levels were quantified as described above; N2 was set at 1 in 4 h (IFNB) or 8 h (ISGs) GW5074-treated HIV-1-infected DCs. Primers are listed in Supplementary Table 2. DC maturation was determined 48 h post infection by flow cytometry analysis of CD80 (575227; BD), CD83 (555658; BD) and CD86 (PN1M2218; Beckmann Coulter) cell surface expression.

**Association of DX3x–MAVS–TRAF3–IRF3 and PLK1.** Whole-cell extracts from HIV-1–infected DCs were prepared 3 h post infection using RIPA buffer (Cell Signaling). DX3x– or MAVS–associated proteins were immunoprecipitated from 40 µg of extract with 4 µg anti-DX3x (sc-98711) or anti-MAVS (sc-68926; both Santa Cruz) coated on protein A/G–PLUS agarose beads (Santa Cruz). Either 20 µg of extract or immunoprecipitate was resolved by SDS–PAGE and detected by immunoblotting with anti-DX3x (1:1,000; 2635; Cell Signaling), anti-MAVS (1:1,000; 3993; Cell Signaling), anti-TRAF3 (1:1,000; 4729; Cell Signaling), anti-IRF3 (1:500; sc-9082; Santa Cruz) and anti-PLK1 (1:1,000; 4513; Cell Signaling), followed by incubation with horseradish peroxidase–conjugated secondary antibody (1:2,500; sc-2314; Santa Cruz) followed by ECL detection (Pierce) (immunoblotting). Rescue of DX3x or MAVS expression after silencing was achieved by transfecting DCs for 48 h with Lys-Coupled–coated (see above) pcDNA3–based expression plasmids containing cDNAs with synonymous mutations that rendered them resistant to the used DX3x and MAVS siRNAs. MAVS cDNA was further modified to encode single Q198K or S409F and dual Q198K/S409F mutants. Plasmids were generated by Life Technologies (ThermoFisher Scientific). Expression of transfected DX3x and MAVS was verified by flow cytometry using anti-DX3x (1:50; 2635; Cell Signaling) and anti-MAVS (1:50; 3993; Cell Signaling) (Supplementary Fig. 6).

**MSM cohort.** Untreated HIV-1–infected MSM participating in the Amsterdam cohort studies (ACS) on HIV-1 infection and AIDS (http://www.amsterdamcohortstudies.org) were genotyped and used for subsequent analyses. Treatment details and exclusion criteria have been described45. ACS are conducted in accordance with ethical principles set out in the declaration of Helsinki and approved by the AMC Medical Ethics Committee; all participants provided written informed consent.

**Virus entry route.** HIV-1 Tat-Rev. Nonspecific Tat activity at 8 h was monitored by transfection of wild-type Tat and Tat-Rev. Nonspecific Tat activity at 8 h was monitored by transfection of wild-type Tat and Tat-Rev. Nonspecific Tat activity at 8 h was monitored by transfection of wild-type Tat and Tat-Rev. Nonspecific Tat activity at 8 h was monitored by transfection of wild-type Tat and Tat-Rev.

**Footnotes:**

1. Natural History Database (NHD; www.naturalhistorydatabase.org).

2. MAbs were purchased from the ATCC and used at dilutions of 1:100 (anti-CD80), 1:50 (anti-CD83), 1:250 (anti-CD86) and 1:1000 (anti-CD80, anti-CD83, anti-CD86) in 4% BSA/PBS.
Human immature Langerhans cells restrict CXCR4-

Association of DDX3 with mitochondrial MAVS was visualized by confocal microscopy. DCs were infected for 3 h; during the last 30 min 200 nM MitoTracker Red CMXRos (Cell Signaling) was added to the medium. Cells were then fixed with 4% paraformaldehyde; permeabilized with 0.2% (vol/vol) Triton X-100 in PBS, and stained with anti-DDX3 (1:50; sc-9871; Santa Cruz), followed by Alexa-Fluor-488-conjugated anti-rabbit Ig (1:400; A21206; Life Technologies), together with 1 µg/ml Hoechst (Invitrogen). Visualization was done with a Leica TCS SP8 X confocal microscope.

Association of PLK1 with either wild-type or mutant MAVS was further examined by capture ELISA. 50 ng Thr210-phosphorylated Flag–PLK1 (Origene; see below) was immobilized on anti-PLK1 (4513; Cell Signaling)-coated high-binding 96-well plates and incubated with whole-cell extracts (see above) from immature DCs from donors with either the minor or major genotype for rs7269203 and rs7269520. MAVS that was associated with PLK1 was detected with anti-MAVS (3993; Cell Signaling), followed by incubation with HRP-conjugated anti-rabbit-IgG (7074; Cell Signaling) and addition of TMB substrate (Cell Signaling). After termination of the coloring reaction, the absorbance was measured at 450 nm.

Activation of TBK1, IKKe, IRF3, PLK1 and MST1. Phosphorylation of TBK1, IKKe, PLK1 and MST1 in HIV-1-infected DCs was detected 1 h (MST1) or 3 h (TBK1, IKKe, PLK1) post infection by flow cytometry. Cells were first fixed in 3% paraformaldehyde for 10 min and then permeabilized in 90% methanol at 4 °C for 30 min. Incubation of the primary antibodies anti-TBK1 p-Ser172 (1:50; 5483; Cell Signaling), anti-IKKe p-Ser172 (06-1340; Millipore), anti-PLK1 p-Thr210 (1:50; 5472; Cell Signaling) or MST1 p-Thr183 (1:50; 3681; Cell Signaling) was followed by incubation with phycoerythrin (PE)-conjugated anti-rabbit-Ig (1:200; 711-116-152; Jackson ImmunoResearch). Phosphorylation was analyzed on a FACS Calibur (BD).

Phosphorylated PLK1 was also detected by immunoblotting. 40 µg of whole-cell extracts (see above) were resolved by SDS–PAGE, and PLK1 was detected by immunoblotting with anti-PLK1 (1:1,000; 4513; Cell Signaling) or anti-PLK1 p-Thr210 (1:1,000; 5472; Cell Signaling), followed by incubation with HRP-conjugated secondary antibody (1:2,500; Cell Signaling). To ensure that equal amounts of protein were used for immunoprecipitation, membranes were also incubated with anti-β-actin (1:2,000; sc-81178; Santa Cruz), followed by HRP-conjugated anti-mouse-IgG (1:1,000; sc-2314; Santa Cruz).

To normalize for RNA input, a sample from each condition before immunoprecipitation was taken; results are expressed as percentage (%) input RNA. To measure the Translation initiation complex composition. Cytoplasmic extracts of HIV-1-infected DCs that were prepared 3 h post infection using ChIP lysis buffer (Active Motif) were treated with micrococcal nuclease (Cell Signaling) for 30 min at 37 °C. Translation initiation complexes were retained on mGTP–agarosa (Jena Bioscience) from 40 µg of nuclease-treated extracts, using the cap analog mGppGpG (New England BioLabs) as a competitor, as previously described, resolved by SDS–PAGE and detected by immunoblotting with anti-DDX3 (1:1,000; 2635; Cell Signaling), anti-MAVS (1:1,000; 3993; Cell Signaling), anti-eIF4G (1:1,000; 2425; Cell Signaling) or anti-PABP (1:1,000; 4992; Cell Signaling), followed by incubation with HRP-conjugated secondary antibody (1:2,500; 21230; Pierce) and ECL detection (Pierce). To ensure that equal amounts of protein were used before mGTP cap binding, membranes were also incubated with anti-β-actin (1:2,000; sc-81178; Santa Cruz), followed by HRP-conjugated anti-mouse-IgG (1:1,000; sc-2314; Santa Cruz).

RNA immunoprecipitation (RIP) assay. RIP and re-RIP assays were performed using EZ-MagnaRIP kit (Millipore). Briefly, DCs were fixed with 1% (vol/vol) paraformaldehyde and lysed. Protein–RNA complexes were immunoprecipitated from the equivalent of 1 million cells, using 2 µg anti-DDX3 (sc-9871; Santa Cruz), anti-MAVS (sc-68926; Santa Cruz), anti-eIF4G (2498; Cell Signaling) or a negative control IgG (sc-2027; Santa Cruz) and protein-G-coated magnetic beads. For re-RIP analyses, DDX3–RNA complexes were eluted from beads and desalted, and a second round of immunoprecipitation was performed with anti-MAVS or anti-eIF4G. RNA–protein cross-links were reversed in 200 mM NaCl for 2 h at 70 °C, before proteinase K treatment for 1 h at 37 °C. mRNA and non-mRNA fractions were separated, isolated and quantified as described, using strict controls to ensure that abortive HIV-1 RNA was measured in non-mRNA samples without cross-contamination with either genomic DNA or mRNA. Primers are listed in Supplementary Table 1.

Erratum: HIV-1 blocks the signaling adaptor MAVS to evade antiviral host defense after sensing of abortive HIV-1 RNA by the host helicase DDX3


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In the version of this article initially published, the fifth author’s surname was spelled incorrectly (as ‘Sarrami-Forooshani’). The correct spelling is ‘Sarrami-Forooshani’. Also, the cells for the third plot in Figure 1d were identified incorrectly as ‘Intestinal DCs’. The correct label is ‘Intestinal DCs’. The errors have been corrected in the PDF and HTML versions of this article.