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# Kinetics of early innate immune activation during HIV-1 infection of humanized mice

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This work is dedicated to the memory of our friend and colleague Marcus Dorner

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**ABSTRACT (216 words)**

Human immunodeficiency virus type-1 (HIV-1) infection is associated with aberrant immune activation, however, most model systems for HIV-1 have been used during established infection. Here, we utilize ultra-sensitive HIV-1 quantification to delineate early events during the HIV-1 eclipse, burst and chronic phases of HIV-1 infection in humanized mice. We show that very early in infection, HIV-1 suppresses peripheral type I interferon (IFN) and interferon-stimulated gene (ISG) responses, including the HIV-1 restriction factor IFI44. At the peak of innate immune activation, prior to CD4 T cell loss, HIV-1 infection differentially affects peripheral and lymphoid TLR expression profiles in T cells and macrophages. This results in a trend towards an altered activation of NFκB, TBK1 and IRF3. The subsequent type I and III IFN responses result in preferential induction of peripheral ISG responses. Following this initial innate immune activation, peripheral expression of the HIV-1 restriction factor SAMHD1 returns to levels below those observed in uninfected mice, suggesting that HIV-1 interferes with their basal expression. However, peripheral cells, still retain their responsiveness to exogenous type I IFN, whereas splenic cells show a reduction in select ISG in response to IFN. This demonstrates the highly dynamic nature of very early HIV-1 infection and suggests that blocks to the induction of HIV-1 restriction factors contribute to the establishment of viral persistence.

**KEYWORDS:** HIV-1, Innate immune activation, humanized mouse

**IMPORTANCE**

Human immunodeficiency virus (HIV)-1 infection is restricted to humans and some non-human primates (e.g. chimpanzee, gorilla). Alternative model systems based on SIV infection of macaques are available but do not recapitulate all aspects of HIV-1 infection and disease. Humanized mice, which contain a human immune system, can be used to study HIV-1 but only limited information on early events and immune responses are available to date. Here, we describe very early immune responses to HIV-1 and demonstrate a suppression of cell-intrinsic innate immunity. Furthermore,

we show that HIV-1 infection interacts differently with innate immune responses in blood and lymphoid organs.

## BACKGROUND

More than 37 million people are currently infected with the human immunodeficiency virus (HIV) (1). Since HIV-1 stably integrates into the host cell genome of CD4 T cells, no curative therapies are currently available (2). The narrow species tropism of HIV-1 limits natural infection to humans, mainly due to incompatibility of host factors and the presence of restriction factors in non-human cells (3, 4). Even though surrogate model systems are available (e.g. simian immunodeficiency virus (SIVmac)), these do not recapitulate all the complex host/pathogen interactions evolved by HIV-1, mostly because they present pre-adaptation strains (i.e. SIVcpz or SIVsm) or HIV-2 progenitors (5, 6). Additionally, SIV and HIV-2 encode an additional accessory protein, Vpx, which alters disease progression by promoting replication in myeloid cells by blocking the action of the restriction factor SAM domain and HD domain-containing protein (SAMHD)-1 (7, 8). Furthermore, *in vitro* models of HIV-1 persistence in T cells generally require pre-activation of the cells, which results in their proliferation and altered phenotype. This is in stark contrast to infection *in vivo*, where any cellular activation is driven by endogenous processes (9). Even though more physiological model systems have been developed, including ex-vivo tissue explant models (10, 11) or cytokine-driven models of HIV-1 latency (12, 13), they often rely on extensive manipulation of natural physiology, are often challenging to accurately control, and have not yet been demonstrated to be equivalent to naturally infected cells.

Humanized mice have been developed to bridge this gap, facilitating HIV-1 infection using human cells *in vivo* (14). These models largely rely on the ability of human hematopoietic stem cells (HSC) to utilize the murine bone marrow stem cell niche to reconstitute highly immunodeficient mice with all major human hematopoietic lineages (15). Even though advanced model systems incorporating implanted human fetal liver and thymus have been developed (16), the limited availability of human

fetal tissue as well as ethical considerations makes human HSC-engrafted humanized mice the most widely used and tractable model (15). Even though many of the humanized mouse models have been used to study HIV-1 infection, currently, no data are available on very early events in the path to HIV-1 persistence. In addition, early innate immune activation by HIV-1 through type I IFN is still poorly understood and even less is known in regard to the role of type III IFN in HIV-1 infection. Furthermore, it remains unclear to what extent HIV-1 infection activates (17-19) or interferes with (20-22) innate immune activation and IFN-based signaling. Even though many ISGs with antiretroviral activity have been described (3, 23), it remains unclear, to what extent these are present, induced, repressed or evaded during HIV-1 infection *in vivo*.

Here, we demonstrate that ultrasensitive detection can be used to distinguish very early eclipse and burst phases preceding the onset of CD4 T cell loss in humanized mice. These phases give clear insight into the differential innate immune activation stages involved in establishing HIV-1 persistence as well as illustrating how HIV-1 subverts host efforts to restrict infection. This offers valuable insight into how HIV-1 interacts with the innate immune system early during infection and explains why common IFN-stimulated HIV-1 restriction factors may be limited in their capacity to control infection.

## METHODS

### Generation of humanized mice

NOD.Cg-*Rag1*$^{tm1Mom}$*IL2rg*$^{tm1Wjl}$/SzJ (NRG) mice were obtained from The Jackson Laboratory and housed and bred at Imperial College London CBS animal facility. Human CD34+ hematopoietic stem cells (HSC) were isolated from human fetal livers as previously described (24). Less than 4-day-old NRG mice were sublethally irradiated with 100cGy and 4 hours later were intrahepatically injected with 1x10$^5$ purified CD34+ HSC's. Each experimental cohort was designed with groups comprising equal engraftment, gender and more than one HSC donor (Table 1).

### HIV-1 infection of humanized mice and interferon treatment

Wild type NL4.3 BaL was generated via transfection in 293T cells then purified on a 20% sucrose cushion prior to injection as previously described (25). Humanized mice cohorts were generated based on equal numbers from different HSC donors, engraftment levels, age and sex prior to infection. Humanized mice were intravenously injected with 2.1IU of reverse transcriptase, as measured by SG-PERT as previously described (26) and mock mice were subsequently injected with an equal volume of vehicle. For the IFNα treated cohorts, HIV-1-infected humanized mice, which displayed stable viraemia, were intraperitoneally injected with 1000IU/g body weight IFNα2 (Invitrogen, Paisley, UK) daily for 5 days and then blood and tissues were immediately harvested.

**Isolation of leukocytes from murine tissues**

Murine peripheral blood was isolated longitudinally using tail-vein bleeding. Peripheral blood mononuclear cells were isolated from whole blood using murine red blood cell lysis buffer (Alfa Aesar, MA, USA) according to the manufacturer's protocol. Fresh tissue isolated from humanized mice was washed in PBS and digested using 0.1% w/v collagenase digestion buffer for 30 minutes at 37°C. The digested tissue was then homogenized through a 70uM cell strainer and loaded onto a Ficoll gradient according to the manufacturer's protocol and the leukocyte 'buffy' layer was washed in PBS and stained for flow cytometry.

**Isolation of RNA from peripheral blood mononuclear cells and splenic tissue**

Fresh splenic tissue was harvested from humanized mice and directly stored in RNAlater stabilization solution (Life Technologies, Carlsbad, CA, USA) and frozen at -80°C. Upon RNA extraction, the samples were thawed and resuspended in the appropriate amount of RLT buffer containing β-mercaptoethanol according to the weight of the tissue or cell number. The tissue was then processed using the TissueLyser LT (Qiagen, Manchester, UK) for 5 minutes at maximum speed. The RNA was processed using Qiashredder columns (Qiagen) followed by direct RNA isolation using the RNeasy mini kit (Qiagen) according to the manufacturer's protocol.

147

148 **Flow cytometry analysis**

149 To evaluate engraftment, 50uL of whole blood was collected from a superficial tail vein 12 weeks
150 following intrahepatic injection and PBMC's extracted as previously described. The isolated cells
151 were then stained using anti-mouse CD45-BV605 (BD Bioscience, NJ, USA) and anti-human
152 CD45-APC (BD) and engraftment measured by the number of human CD45+ cells in the total
153 leukocyte population.
154 Evaluation of human cell populations during both reconstitution analysis in organs and HIV-1
155 infection studies were performed as described above and staining also included anti-human CD3-
156 PerCP Cy5.5 (BD), anti-human CD4-APC-H7 (BD), anti-human CD8-AF700 (BD), anti-human
157 CD14-BV510 (BD), anti-human CD19-PE (BD), anti-human CD11b-BV421 (BD), anti-human
158 IFNAR1-PerCP (Novus, Manchester, UK), anti-human IL28RA-PE (Biolegend, CA, USA), anti-
159 human BST2 (BioLegend) and the Live/Dead Fixable Dead cell staining (Life Technologies).
160 Additionally, intracellular staining was performed using the BD Cytofix/Cytoperm solution kit
161 (BD) following the manufacturer's protocol using the following antibodies; KC57-p24 (Beckman
162 Coulter, CA, USA), anti-human TLR3-APC (Miltenyi, Bergisch Gladbach, Germany), anti-human
163 TLR7-Alexa405 (R&D Systems, MN, USA), anti-human TLR8-Alexa350 (R&D) and anti-human
164 TLR9-FITC (Abcam, Cambridge, UK). For absolute cell counts, CountBright Absolute Counting
165 beads for flow cytometry (ThermoFisher MA, USA) were added to each sample according to the
166 manufacturer's protocol. All data was analysed using FlowJo software.

167

168 **Phosflow analysis of protein phosphorylation**

169 Cells isolated from humanized mice were prepared for flow cytometry as described above and
170 stained with the following extracellular markers; anti-mouse CD45-BV605 (BD), anti-human
171 CD45-APC (BD), anti-human CD3-APC-eFluor780 (ThermoFisher), anti-human CD8-AF700
172 (BD), anti-human CD33-BV711 (BD). Cells were fixed with Cytofix buffer (BD) at 37°C for 10

minutes. Following this, cells were permeabilized with Permeabilisation buffer III (BD) for 30 minutes at 4°C and stained with the following phosflow antibodies; anti-human pSTAT1-BV421 (BD), anti-human pp65-PerCP eFluor710 (Life Technologies), anti-human pIRF3 S396-PE (Cell Signalling, MA, USA) and anti-human pTBK-1 (Cell Signalling).

**HIV-1 ultra-sensitive RNA quantification**

Total RNA was isolated from <50uL murine plasma-EDTA using the QIAmp viral RNA isolation kit (Qiagen) according to the manufacturer's protocol. In the first round, extracts were amplified using a semi-nested RT-PCR with a limited cycle number using the Superscript III One-Step RT-PCR system (Invitrogen) with primers (SID1: 5'-AAGACAGCAGTACAAATGGCAGT-3' and SID2: 5'-TACTGCCCCTTCACCTTTCCA-3') targeting the HIV-1 integrase genomic region. The internal control RNA is a transcript of the integrase gene with the probe-binding region containing a randomized sequence of 25 nucleotides. The second round uses the subsequent DNA product from the semi-nested RT-PCR as template for the qPCR and HIV copies detected using the QuantiTect Probe PCR kit (Qiagen) using the following primers and probes (SID2, SID3: 5'-CAATTTTAAAAGAAAAGGGGGGATT-3', HIV-1 probe: 5'-FAM–CGGGTTTATTACAGGGACAGCAGA–TAMRA-3' and internal control probe: 5'-VIC–CTGGGTAGAGTAGTCACAGAATGCG– BHQ-3').

**Gene expression analysis**

Isolated RNA extracted from cells or tissue was converted into cDNA using High capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, USA) following the manufacturer's protocol. Quantification of human IFNα, IFNβ, IFNλ3 and IFNλ1 and ISG expression SAMHD1 (5'-TCACAGGCGCATTACTGCC-3', 5'-GGATTTGAACCAATCGCTGGA-3'), myxovirus resistance protein (MX)-1, MX2 (5'- CAGCCACCACCAGGAAACA-3', 5'-TTCTGCTCGTACTGGCTGTACAG-3'), IFITM1 and IFI44 were evaluated using SYBR Green

PCR mastermix (Life Technologies) on the ViiA™ 7 Real-Time PCR System instrument (Applied Biosystems) using previously described primer sequences (27). Subsequent mRNA levels were normalized to GAPDH (5'-AGGGCTGCTTTTAACTCTGGT-3', 5'-CCCCACTTGATTTTGGAGGGA-3') expression. All primers were generated through Life Technologies.

**Statistical analysis**

All statistical analyses were performed using GraphPad Prism v6.0 and data were evaluated for statistical significance between experimental cohorts and conditions using one phase exponential decay with least squares test, ordinary One-way ANOVA with Bonferroni's multiple comparison test. For the comparison of mock-infected with HIV-1-infected animals, the median-based unpaired Mann-Whitney test was performed with Bonferroni corrections. In contrast, a comparison of the same HIV-1 infected humanized mice at different time-points was analysed using the median-based paired Wilcoxon matched-pairs ranked sign test with Bonferroni corrections. For each statistical analysis, a $p$ value of $<0.05$ was considered significant.

**Safety/Biosecurity**

All work with infectious agents was conducted in biosafety level 3 facilities, approved by the Health and Safety Executive of the UK and in accordance with local rules, at Imperial College London, UK.

**Statement on animal ethics**

All work was approved by the local genetic manipulation (GM) safety committee of Imperial College London, St. Mary's Campus (centre number GM77), and the Health and Safety Executive of the United Kingdom and carried out in accordance with the approved guidelines. All animal research described in this study was approved and carried out under a United Kingdom Home

Office License, PPL 70/8219 in accordance with the approved guidelines, under the Animals (Scientific Procedures) Act 1986 (ASPA).

## RESULTS

### HIV-1 infection exhibits a multiphasic kinetic, stabilizing after three weeks

Despite many studies utilizing humanized mice to model HIV-1 infection (15), little information was available on very early virological and immunological events following infection. The generation of humanized mice by intrahepatic injection of HSC into sub-lethally irradiated NRG mice resulted in multilineage engraftment of human immune cells in all major organs within three months (Figure 1a-b). This included $CD3^+$ T cells, $CD19^+$ B cells as well as lineage-negative cells (Figure 1c). $CD3^+CD4^+$ T helper cells, the main target for HIV-1 infection, as well as $CD3^+CD8^+$ cytotoxic T cells, were present in physiological frequencies and, despite their lower repopulation, both, $CD11b^+$ macrophages and $CD14^+$ monocytes were present in all major organs (Figure 1d). Infection of these mice with the CCR5-tropic HIV-1 recombinant virus, NL4.3 (BaL env), resulted in stable viremia, as determined by ultrasensitive qRT-PCR (Figure 2a). Notably, this method allowed us to observe three phases during infection; an eclipse phase up to day 4 followed by a burst phase of viral replication before expansion of HIV-1 RNA copies in the plasma of mice from 4 to 9 days before stable peak viremia was reached (Figure 2a). Non-humanized control cohorts were utilized to demonstrate rapid virological decay in the absence of human cells (Figure 2b). Strikingly, when comparing the initial decay of HIV-1 RNA in the serum of HIV-1-infected humanized and non-humanized mice within the first three days of the eclipse phase, the serum HIV-1 RNA half-life was significantly prolonged in mice containing human immune cells (Figure 2c). At day 22 post-infection, *de novo*-produced infectious HIV-1 particles were detectable in the serum of infected mice as determined by titration on GHOST cells (Figure 2d). p24-expressing HIV-1-infected $CD3^+CD4^+$ T cells were detectable with a peak at 7 days post-infection before contracting to stable levels, detected in both peripheral blood and splenic tissue of these mice (Figure 2e-g).

This shows that HIV-1 infection in humanized mice can be assessed at very early time-points post infection and that HIV-1 viremia initially contracts prior to establishing stable serum viremia.

Among the hallmarks of HIV-1 infection is the resulting longitudinal loss of CD4 T cells (28). Critically, and similar to HIV-1 infection in humans, in this model HIV-1 infection resulted in an overall reduction of total CD4 T cell counts in peripheral blood and spleen (Figure 2h-j). This progressively worsened, and in some respects therefore recapitulated progression to AIDS in human infection (Figure 2h-j). As expected, the infection perturbs the total human leukocyte engraftment when compared to uninfected mice, leaving the murine CD45-expressing leukocyte population unchanged (Figure 2k). This demonstrates that the trajectories of HIV-1 infection in humanized mice bear similarities to those observed in humans.

**HIV-1 distinctly suppresses early innate immune activation**

Reports from early HIV-1 infection in patients suggest a strong peripheral type I IFN response to infection (29). However, little information was available on the kinetics and the correlation of peripheral circulation with lymphoid responses. Thus, it remained unclear as to whether HIV-1 is able to suppress early host responses. Since HIV-1 infection is associated with immune hyperactivation (30), we aimed to measure the extent of innate immune activation throughout the establishment of HIV-1 persistence in humanized mice. To this end, we evaluated the induction of type I and III IFN at early, intermediate and late time-points. Interestingly, at only 24 hours post infection, HIV-1 specifically downregulates type I IFN (IFNα and IFNβ) expression in peripheral blood lymphocytes (Figure 3a-b), while neither of the type III IFNs were expressed (Figure 3c-d). During the viral eclipse phase up to day 7, HIV-1 infection was associated with marked elevation of both, type I and type III IFN in peripheral blood lymphocytes (Figure 3a-d). Once HIV-1 infection was fully established at day 35 post infection, type I and III IFN return to baseline levels in the peripheral circulation (Figure 3a-d).

To evaluate, what mechanism drives the observed peripheral and splenic production of type I and III IFN at the peak of innate immune activation, prior to the HIV-1-associated depletion of CD4 T

277 cells or macrophages (Figure 4a, d), we evaluated the presence of pattern recognition receptors
278 (PRR) able to sense viral RNA on peripheral as well as splenic CD4 T cells (Figure 4b, c) and
279 macrophages (Figure 4e, f) 10 days following infection with HIV-1. At this point, HIV-1 infection
280 resulted in a trend towards suppression of Toll-like receptor (TLR)3 protein expression on splenic,
281 but not peripheral CD4 T cells, whereas peripheral TLR7 and TLR9 protein expression was
282 elevated after HIV-1 infection of both, CD4 T cells and macrophages (Figure 4b, c, e, f).
283 To determine, whether this differential TLR profile may be involved in recognition of HIV-1
284 infection, we performed phosflow pathway analysis of nuclear factor (NF)-κB, TANK-binding
285 kinase (TBK)1 and interferon regulatory factor (IRF)3 in peripheral and splenic CD4 T cells and
286 macrophages 10 days following infection of humanized mice with HIV-1 (Figure 4g-m). While
287 splenic CD4 T cells respond to HIV-1 infection by inducing phosphorylation of the NFκB subunit
288 p65 at serine 536, this was not observed in peripheral CD4 T cells or macrophages (Figure 4h, k).
289 Even though many PRR pathways involve the phosphorylation of TBK1, only macrophages, but
290 not CD4 T cells exhibited elevated mean fluorescence intensities of phosphorylated TBK1 (Ser172)
291 (Figure 4i, l). Peripheral but not splenic CD4 T cells additionally showed a trend towards elevated
292 levels of phosphorylated IRF3 (Ser396) (Figure 4j, m).
293 Taken together, this indicates that the HIV-1-induced induction of TLR7 and TLR9 expression in
294 PBMC may be at least partially responsible for peripheral interferon responses.
295
296 **HIV-1-associated early immune activation is driven by both, T cells and macrophages in**
297 **peripheral, but not lymphoid tissue.**
298 Since HIV-1 infection resulted in a very compartmentalized, early IFN response in PBMC but not
299 splenic tissue, we assessed the distribution of the respective receptors for type I and III IFN 10 days
300 following HIV-1 infection by flow cytometry (Figure 5a-d). As expected, the majority of
301 macrophages in either PBMC or spleen expressed either IFNAR1, IL28RA or IFNAR1 and

IL28RA (Figure 5b, d), whereas only a minority of CD4 T cells expressed predominantly IFNAR1 but not IL28RA (Figure 5a, c).

To assess, which cell populations responded to production of type I and III IFN, we next examined the level of STAT1 phosphorylation at tyrosine 701, which is indicative of activated JAK/STAT signaling downstream of type I and III IFN receptors (Figure 5e, f). This showed that macrophages in PBMC, but not in spleen exhibited activated JAK/STAT signaling in response to HIV-1 infection, indicative of the observed production levels of type I and III IFN (Figure 5e, f). This also directly correlated with the production of ISG, as exemplified by tetherin/BST2, which was preferentially induced by HIV-1 infection in CD4 T cells and macrophages in peripheral circulation, but was not induced in splenic CD4 T cells or macrophages.

This compartmentalized IFN response additionally translated to the selective induction of well-known ISG and HIV-1 restriction factors, including MX2, IFITM1, and IFI44 in PBMC at the peak of the initial HIV-1 replication burst (Figure 6a-e). Surprisingly, however, both, at the eclipse phase of infection as well as during late-stage chronic infection, HIV-1 infection suppressed baseline levels of IFI44 and showed similar trend with SAMHD1 and MX2 expression, indicating that HIV-1 actively shapes its environment.

Taken together, this demonstrates that during early establishment of HIV-1 persistence in this model, innate immune activation in different compartments is highly dynamic, exhibiting signs of HIV-1-associated innate immune evasion and repression of expression of key HIV-1 restriction factors.

**Exogenous type I IFN can override HIV-1-induced repression of restriction factors only in peripheral circulation**

Based on recent experimental studies of SIV infection of macaques, it is possible that type I IFN accelerates HIV-1 disease progression (31, 32). However, details on the responsiveness of HIV-1 infected cells to IFN and the subsequent expression of IFN-induced restriction factors are still

328 lacking. To evaluate, whether chronic HIV-1 infection durably suppresses the induction of IFN
329 responses and HIV-1 restriction factors, we treated HIV-1-infected humanized mice with 1,000
330 IU/g/day exogenous recombinant (r)IFNα for five consecutive days. This treatment resulted in the
331 significant reduction of circulating serum HIV-1 RNA levels (Figure 7a, b), indicating that the
332 elicited inflammatory responses partially inhibited HIV-1 replication. Notably, administration of
333 rIFNα in HIV-1-infected humanized mice exacerbated the observed HIV-1-associated CD4 T cell
334 depletion, indicating that ISG and proinflammatory cytokines induced by type I IFN contribute to
335 HIV-1-associated pathogenesis (Figure 7d).
336 As expected, neither IFNα, IFNβ nor IFNλ1 mRNA expression was elevated by rIFNα (data not
337 shown), whereas IFNλ3, which is itself IFN-stimulated, was induced by 3 log10 at the end of
338 treatment (Figure 7e, j). rIFNα treatment resulted in the statistically significant increase of both,
339 SAMHD1 and MX2 in PBMC of HIV-1-infected humanized mice, whereas IFITM1 and IFI44
340 remained unchanged (Figure 7f-i). Notably, despite a more than 2 log10 induction of IFNλ3 in
341 response to rIFNα treatment in splenocytes of HIV-1 infected humanized mice, HIV-1 restriction
342 factors, including SAMHD1, MX2, IFITM1 and IFI44 remained unchanged or were down-
343 regulated by rIFNα (Figure 7k-n). This indicates that HIV-1 infection does not result in suppression
344 of IFN responsiveness in peripheral circulation but induces a compartmentalized interference with
345 the induction of HIV-1 restriction factors in lymphoid tissue.
346
347 **DISCUSSION**
348 Chronic HIV-1 infection is associated with immune hyperactivation. Even though the kinetic of
349 natural HIV-1 infection has been extensively studied in the past, limited information is available on
350 very early events following infection. Even though surrogate models for HIV-1 infection have been
351 used in the past to dissect this, vast genetic differences as well as the presence of the SIV accessory
352 protein Vpx makes it difficult to directly correlate data from non-human primates with HIV-1
353 infection in humans. The main challenge in utilizing human samples to study early events in HIV-1

354 infection are nearly always limited to peripheral blood, making it impossible to translate findings to
355 events within lymphatic systems, as well as logistical challenges associated with specimen
356 collection during the initial phases of HIV-1 infection.
357 Utilizing humanized mice, we show that HIV-1 infection and subsequent persistence is established
358 as early as 4 days post infection, as evidenced by comparing HIV-1 serum RNA load to mice
359 without humanization. This far precedes the significant loss of CD4 T cells, which only becomes
360 notable after one month of infection.
361 Strikingly, HIV-1 is able to suppress baseline peripheral IFN and ISG levels very early following
362 infection, before HIV-1 RNA levels becomes detectable in peripheral circulation. This early
363 suppression may contribute to the spread of HIV-1-infected cells, since key HIV-1 restriction
364 factors such as SAMHD1 and MX2 are present in host cells at lower levels. Only one week
365 following infection, when serum HIV-1 RNA is already detectable, there is a significant peripheral
366 type I and III IFN signature. However, the lymphatic system, as evidenced by measurements in the
367 spleen, does not experience type I IFN production and instead, exhibits a type III IFN signature.
368 This absence of splenic type I IFN responses coincides with a trend towards reduced expression of
369 the RNA sensor TLR3 on splenic CD4 T cells. Additionally, in contrast to peripheral CD4 T cells,
370 HIV-1 infection does not induce expression of TLR7 in splenic CD4 T cells or macrophages. This
371 suggests a skewing of HIV-1-induced signaling towards pro-inflammatory NFκB, rather than IFN-
372 inducing IRF3 signaling in T cells and macrophages in the spleen. This could contribute to the
373 pyroptosis-induced CD4 T cell loss characteristic during HIV-1 infection (33). In contrast,
374 peripheral CD4 T cells show a trend towards increased phosphorylation and activation of IRF3.
375 This may, at least in part, explain the observed differential and compartmentalized type I and III
376 IFN production.
377 This is furthermore underpinned by the absence of an ISG response in the spleen, whereas multiple
378 ISG and HIV-1 restriction factors are upregulated on the mRNA and protein level in the peripheral
379 circulation. This absence of early IFN responses explains why HIV-1 exhibits an early reservoir

expansion in the near absence of restriction by host factors. Notably, even after the early peripheral induction of IFN and ISG responses, persistence of HIV-1 is associated with reduced expression of select ISG and restriction factors in peripheral blood, which further underpins the ability of HIV-1 to evade host restriction. This suppression of key HIV-1 restriction factors may render the host more susceptible to systemic dissemination of HIV-1 infection.

Despite this evasion mechanism, PBMC retain their responsiveness to type I IFN, as exogenous treatment with recombinant IFNα results in the induction of SAMHD1, IL28b and MX2. However, splenic cells of HIV-1-infected humanized mice, upon treatment with recombinant IFNα display an inverse response to type I IFN, resulting in repressed restriction factor expression. In particular SAMHD1 is significantly downregulated after IFN treatment, suggesting that HIV-1 may have evolved means to facilitate its dissemination in lymphatic tissue despite immune activation. This cannot be explained by a potential hurdle for rIFNα to penetrate to the spleen upon exogenous administration, since IL28b, which itself is IFN-stimulated is readily detectable in the spleen. Surprisingly, another study using BLT mice, which exhibit a more functional adaptive immune system with the T cells' ability to mount protective antigen-specific immune responses has demonstrated that CD4 T cell loss is delayed and that MX1 levels are chronically elevated (34). We observe very similar elevations of MX1, however, MX1, in contrast to MX2 has not been shown to restrict HIV-1 infection and a comparative analysis in humanized mice has not been previously performed (35). Thus, the different kinetics of CD4 T cell loss may be due to antigen-specific T cell responses against HIV-1 infection releasing IFNγ. Additionally, another study has demonstrated that blocking type I IFN signaling results in T cell recovery during HIV-1 infection, supporting the notion that type I IFN might be associated with disease progression (36).

Taken together, we show that IFN and ISG responses during acute HIV-1 infection in humanized mice are highly dynamic, exhibiting evidence for early viral suppression and peak recognition of HIV-1 by the innate immune system during the initial burst of HIV-1 replication. The trend observed indicating reduced TLR3 expression on splenic CD4 T cells upon HIV-1-infection could

additionally contribute to the lack of HIV-1 RNA recognition. Since most of the initially induced HIV-1 restriction factors are returning to baseline or below baseline expression during chronic HIV-1 infection, this could explain why HIV-1 is largely uncontrolled at later stages during infection. The induction of other ISGs without direct antiretroviral action could potentially result from other activation pathways and do not necessarily reflect the responses of ISGs in general. Indeed, CXCL10, which is commonly used as marker for IFN stimulation and was shown to be elevated in untreated HIV-1-infected patients (37) was recently shown to be induced via TLR7/9 activation (38), rather than through JAK/STAT signaling. Most strikingly, the observed very early suppression of IFN and ISG responses *in vivo* has not been observed thus far. This early interference of pathogen sensing and IFN activation by HIV-1 may function as a viral strategy to establish an early reservoir and to eliminate restriction factor roadblocks preventing the establishment of HIV-1 persistence. The mechanism, by which HIV-1 infection results in the transcriptional down-regulation or destabilization of IFN mRNA remains elusive. However, many host proteins as well as viral accessory proteins (e.g. Vpr) are directly packaged within the HIV-1 virion and could contribute to this early down-modulation of the IFN system. This suggests that careful future analysis of single cell transcriptional responses *in vitro* and *in vivo* is required to delineate, which restriction factors are present and functional in HIV-1-infected as well as –uninfected bystander cells.

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**Figure legends**

**Figure 1. Humanized mice engraft with human cells in all major organs.**

(a) Representative flow cytometry plots depicting human cell populations in humanized (HIS) NRG mice in both the peripheral blood and spleen. (b) Percentage of human cell engraftment in HIS mice in the PBMC, spleen, brain, lung and liver tissue. Engraftment is depicted as the percentage of human CD45+ cells in the total leukocyte population (e.g. human CD45 and mouse CD45). (c) Frequency of CD3+ T cells, CD19+ B cells and lineage negative cells ($Lin^-$, CD3-CD19-) in PBMC, spleen, brain, lung and liver. (d) Frequency of CD4+ and CD8+ T cells (within the CD3+ cell population) and CD14+ monocytes and CD11b+ macrophages (within the lineage negative population) as depicted in 1c. Data shown are from 12 humanized NRG mice reconstituted with 3 different CD34 HSC donors.

**Figure 2. HIV-1 results in distinct phases of infection in humanized mice.**

(a) Serum HIV-1 RNA kinetic in humanized NRG mice following infection with 2.1 IU RT NL4.3 (BaL env) as measured by two-step qRT-PCR. Data shown are mean ± SEM (blue circles) of 12 mice per cohort as well as individual animals (grey lines). Numbers indicate eclipse (1), burst (2) and chronic (3) phases of infection. (b) Serum HIV-1 RNA kinetic in non-humanized NRG mice following infection with 2.1 IU RT NL4.3 (BaL env) as measured by two-step qRT-PCR. Data shown are mean ± SEM (blue circles) of 7 mice per cohort as well as individual animals (grey lines). (c) Serum HIV RNA half-life calculated between day 1 and 3 of infection of HIS-NRG or NRG mice by one phase exponential decay least squares fitting. (d) HIV-1 infectious units in plasma isolated from HIV-1-infected humanized mice at day 22-post infection as measured by GHOST cell titration. (e) Representative flow cytometry plots of CD3+ CD4+ p24+ T cells in both HIV-1 infected and uninfected HIS mice in peripheral circulation and splenic tissue. (f, g) Percentage of HIV-1-infected CD3+ CD4+ p24+ cells observed in (f) PBMC and (g) splenic tissue at days 10 and 35-post HIV-1 infection as determined by flow cytometry and analysed by Wilcoxon matched-pairs signed rank test with Bonferroni corrections. (h) Longitudinal CD4 T cell loss in peripheral blood of HIV-1-infected HIS mice. Data shown are mean ± SEM (blue circles) of 5 mice per cohort as well as individual animals (grey lines). Statistical significance was determined between mock-infected and HIV-1-infected humanized mice at day 49 post infection using Mann-Whitney with Bonferroni corrections. (i, j) Absolute CD4 T cell loss in (i) PBMC and (j) spleen of HIS mice 35 days following infection with 2.1 IU RT NL4.3 (BaL env). Data shown are mean ± SEM (blue circles) of 19 and 9 mice per cohort, respectively, analysed by Mann-Whitney test using Bonferroni corrections. (k) Cell count of human and mouse CD45 leukocytes in HIS mice before and 35 days after HIV-1 infection. Data shown are mean ± SEM of 9 uninfected and 10 HIV-1-infected mice Wilcoxon matched-pairs signed rank test with Bonferroni corrections. For all statistical tests, a $p$ value of $<0.05$ was deemed significant.

**Figure 3. HIV-1 induces a compartmentalized interferon response in humanized mice.** (a-d) mRNA expression of (a) IFNα, (b) IFNβ, (c) IFNλ3 and (d) IFNλ1 in peripheral blood lymphocytes and splenic lymphocytes isolated from HIV-1-infected humanized mice at 1, 7 and 35 days and 10 and 35, respectively. Statistical significance was determined between mock and HIV-1 infected mice using Mann-Whitney test with Bonferroni corrections. Data shown are mean ± SEM of 5 mice per cohort for peripheral lymphocytes and 3 uninfected and 4 HIV-1-infected mice for splenic lymphocytes.

**Figure 4. HIV-1 infection regulates TLR expression and induces differential pattern recognition in T cells and macrophages.** (a) Total human CD4 T cell count in PBMC and spleen of uninfected and HIV-1-infected humanized mice 10 days following infection as determined by flow cytometry. (b) Representative flow cytometry plots and (c) combined protein expression of TLR3, TLR7, TLR8 and TLR9 in human CD4 T cells from PBMC and spleen of uninfected and HIV-1-infected human CD4 T cells 10 days following infection as determined by flow cytometry and analysed by Mann-Whitney test with Bonferroni corrections. (d) Total human CD33+ macrophage counts in PBMC and spleen of uninfected and HIV-1-infected humanized mice 10 days following infection as determined by flow cytometry. (e) Representative flow cytometry plots and (f) combined protein expression of TLR3, TLR7, TLR8 and TLR9 in human CD33+ macrophages from PBMC and spleen of uninfected and HIV-1-infected human CD4 T cells 10 days following infection as determined by flow cytometry and analysed by Mann-Whitney test with Bonferroni corrections. (g) Representative flow cytometry plots and (h-m) combined frequency and mean fluorescence intensity of protein phosphorylation of (h, k) p65 Ser536 (NFκB), (i, l) pTBK1 Ser172 and (j, m) pIRF3 Ser396 in (h-j) human CD4 T cells and (k-m) human CD33+ macrophages in PBMC and spleen of uninfected and HIV-1-infected humanized mice 10 days following infection as determined by flow cytometry and analysed by Mann-Whitney test with Bonferroni corrections.

Data shown are mean ± SEM of 6 uninfected and 7 HIV-1-infected mice. For all statistical tests, a *p* value of <0.05 was deemed significant.

**Figure 5. HIV-1 infection induces JAK/STAT signaling in human T cells and macrophages in humanized mice.** (a, b) Mono- and co-expression of IFNAR1 and IL28RA on (a) human CD4 T cells and (b) human CD33 macrophages in PBMC and spleen of uninfected and HIV-infected humanized mice 10 days following infection. (c, d) Phosphorylation of STAT1 (Tyr701) in (c, d) Expression of IFNAR1 and/or IL28RA on (c) CD4 T cells and (d) macrophages in PBMC and spleen of uninfected and HIV-1-infected humanized mice 10 days following infection as determined by flow cytometry. (e, f) Frequency and mean fluorescence intensity of phosphorylated STAT1 at tyrosine 701 in (e) human CD4 T cells and (f) macrophages in PBMC and spleen of uninfected and HIV-infected humanized mice 10 days following infection as determined by flow cytometry and analysed by Mann-Whitney test with Bonferroni corrections. (g, h) Cell surface expression of BST2/tetherin on (g) human CD4 T cells and (h) macrophages 10 days following infection as determined by flow cytometry and analysed by Mann-Whitney test with Bonferroni corrections. Data shown are mean ± SEM of 6 uninfected and 7 HIV-1-infected mice. For all statistical tests, a *p* value of <0.05 was deemed significant.

**Figure 6. Dynamic gene expression profiles of key HIV-1 restriction factors indicates early expression is perturbed**

(a-f) mRNA expression of (a) SAMHD1, (b) MX1, (c) MX2, (d) IFITM1, and (e) IFI44 in peripheral blood lymphocytes and splenic lymphocytes isolated from HIV-1-infected humanized mice at 1, 7 and 35 days and 10 and 35, respectively. Data shown are mean ± SEM of 5 mice per cohort for peripheral lymphocytes and 3 uninfected and 4 HIV-1-infected mice for splenic

lymphocytes. Statistical significance was determined for unpaired mock and HIV-1 infected mice using Mann-Whitney test, paired HIV-1 infected mice between days 1,7, and 35 using Wilcoxon matched-pairs signed rank test, both with Bonferroni corrections. For all statistical tests, a *p* value of <0.05 was deemed significant.

**Figure 7. HIV-infected peripheral but not splenic cells retain their responsiveness to exogenous interferon.** (a) Longitudinal serum HIV-1 RNA copies in humanized mice upon treatment with 1,000 IU/g rIFNα intraperitoneally for 5 consecutive days (red shading). (b) Serum HIV RNA copies in untreated and rIFNα-treated mice 5 days after treatment as determined by HIV qRT-PCR and analysed by Mann-Whitney test with Bonferroni corrections. (c) Number of p24-positive CD4 T cells in PBMC and spleen of untreated and rIFNα-treated mice 5 days after treatment as determined by flow cytometry. (d) CD4 T cell count in PBMC and spleen of untreated and rIFNα-treated mice 5 days after treatment as determined by flow cytometry and analysed by Mann-Whitney test with Bonferroni corrections. (e-n) mRNA expression of (e, j) IL28b, (f, k) SAMHD1, (g, l) MX2, (h, m) IFITM1 and (i, n) in (e-i) PBMC and (j-n) spleen of untreated and rIFNα-treated mice 5 days after treatment as determined by qRT-PCR and analysed by Mann-Whitney test with Bonferroni corrections. Data shown are mean ± SEM of 5 mice per cohort and for all statistical tests, a *p* value of <0.05 was deemed significant.

659 **Tables**

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661 **Table 1. Humanized mouse cohort information**

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| | Mock infection | HIV infection | Total |
|---|---|---|---|
| Number of mice | 41 | 55 | 96 |
| Animal sex [m/f] | 18/23 | 21/34 | 96 |
| HSC donors | 10 | 12 | 12 |

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