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Interferon- α drives monocyte gene expression in chronic unsuppressed HIV-1 infection

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Introduction

Human immunodeficiency virus (HIV-1) infection severely impacts the immune system causing phenotypic changes in peripheral cells altering both innate and adaptive immune responses. The typical consequence of untreated HIV-1 infection is AIDS resulting from direct infection of susceptible cells and indirect immune activation [1]. Within weeks of infection, during the acute phase, HIV-1 attacks and kills CCR5-expressing CD4⁺ T lymphocytes in the gastrointestinal tract, which impairs the mucosal layer and integrity of the barrier function [2]. The damaged GI tract permits the translocation of bacteria and bacterial products, including lipopolysaccharide (LPS), which is a major structural component of the outer wall of Gram-negative bacteria and is elevated in HIV-1-infected subjects [2]. Lipopolysaccharide in the plasma interacts with LPS-binding protein and subsequently binds cell surface receptor CD14 prior to transfer to the toll-like receptor 4 (TLR-4) and MD2, a secreted glycoprotein required for LPS binding to TLR-4 [3]. Peripheral monocytes express surface markers CD14 and TLR-4, are mononuclear cells in blood that respond to LPS. Elevated levels of LPS in the periphery are associated with increased soluble CD14 (sCD14) [4] and are sufficient to induce an acute-phase inflammatory response [5].

The innate immune system responds immediately following HIV-1 infection producing cytokines designed to limit viral infection and replication. Interferon- α is an early response factor whose role in HIV-1 infection remains a paradox having attributes of both disease protection and progression (review [6]). In both humans and non-human primates, elevated IFN- α is associated with acute HIV-1 and SIV infection, respectively [7,8]. In individuals infected with HIV-1, viral replication correlated with upregulation of type I interferon-stimulated gene expression profiles [9]. Early studies suggested a modest therapeutic effect of IFN- α by preventing CD4 decline and reducing the incidence of AIDS-associated opportunistic infections [10]. However, later research implicated IFN- α in spontaneous apoptosis of uninfected CD4 T cells [11], T-cell depletion [12] and disease progression [13].

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In another study, the adverse effects of IFN- α were demonstrated in a HIV-infected population immunized to IFN- α where individuals with elevated anti-IFN- α antibodies experienced significantly lower rates of HIV-1-related events [14,15]. So while IFN- α effectively retards virus infection and limits virus production at the cellular level, it may be responsible for impairing the immune system and chronic immune activation.

Monocytes are key immune responsive cells whose function is adversely impacted by HIV-1. Unlike activated CD4+ T cells, monocytes are recalcitrant to infection [16] requiring maturation to a macrophage to be highly susceptible to infection. For subjects on highly active antiretroviral therapy (HAART), estimates of less than 1% of monocytes harbor replication-competent virus with no indication that infection progresses to cell death [17]. Despite low rates of infection, monocytes are impaired during viremia exhibiting diminished production of proinflammatory cytokines IL-1 β , IL-6 and TNF- α [9]. Previously, we reported that microarray analysis of freshly isolated monocytes from HIV-1-infected subjects with high viral loads (HVL, >10,000 RNA copies/ml) expressed an altered phenotype with macrophage markers [18].

In this study, we examined the gene expression of freshly isolated CD14+ monocytes from HIV-1-infected patients, who were either successfully treated with HAART with low viral loads (LVL, <10,000 RNA copies/ml) or from subjects with HVL due to HAART failure or treatment interruption. Segregating the monocyte gene expression data based on subject viral load revealed an expression profile indicating that unsuppressed viremia is a significant factor impacting monocyte phenotype. Since CD14+ monocytes are typically responsive to type 1 and 2 IFNs, and LPS, we evaluated the activation status of monocytes in HIV-1-infected patients by gene expression analysis. We then compared the patient monocyte gene expression profiles with expression data from HIV-1-seronegative CD14+ monocytes that were treated *in vitro* with IFN- α , IFN- γ or LPS. When monocyte microarray results from HVL subjects were compared with the *in vitro*-treated monocytes, they displayed an IFN- α activation profile. In addition, there was no evidence of an LPS-induced gene expression profile even though LPS and sCD14 were elevated in the plasma of HIV-1-infected subjects. The implication of these findings is that IFN- α reprograms the innate immune response of monocytes and desensitizes them to normally activating microbial factors.

Materials and Methods

Participants

This study was conducted with the participation of individuals recruited at the San Francisco VA Medical Center with written consent approved by the University of California, San Francisco Committee on Human Research. Global gene expression analysis using high-density cDNA microarrays was performed on CD14+ monocytes isolated from 55 subjects, 22 with HIV-1 HVL, 22 with HIV-1 LVL and 11 HIV-1 seronegative controls. The categorization of high or low viral load was based on clinical criteria with LVL <10,000 RNA copies/ml and HVL as >10,000 RNA copies/ml. The LVL group included 14 subjects with undetectable viral loads (<50 RNA copies/ml). Subjects in the study were males between 30 and 66 years of age with the mean age for control subjects being 53 years $\pm$ 4.1 ($\pm$ SD), the mean age of LVL subjects being 51 years $\pm$ 8.3 and the mean age of HVL subjects being 50 years $\pm$ 7.7. The cohort was comprised of white (62%), black (19%), Hispanic (12%), Asian (4%) and other (3%) individuals. At the time of the study, individuals in the LVL group were on HAART, while subjects with HVL were in one of three categories: on HAART (n=15); scheduled treatment interruption (n=6) or HAART naïve (n=1). When monocytes were isolated for gene expression analysis, median CD4 counts for the groups were: controls = 1038 cells/ μ l; LVL = 429 cell/ μ l and HVL = 123.5 cell/ μ l and a Student's t-test revealed a statistically significant difference in CD4 counts

between LVL and HVL ($P=0.0015$). No significant difference was detected in median nadir CD4 counts between LVL = 146.5 cells/ μ l and HVL = 52.5 cells/ μ l ($P=0.187$).

Isolation of CD14+ monocytes

Whole blood was drawn into Vacutainer CPT tubes (BD, Franklin Lakes, NJ) and peripheral blood mononuclear cells (PBMC) were enriched by centrifugation at $1,600 \times g$ for 25 min (27). CD14+ monocytes were positively selected from PBMC immunomagnetic separation using anti-CD14 monoclonal antibodies conjugated to ferrous beads according to the manufacture's instructions (Miltenyi Biotec, Auburn, CA). On average, 3×10^6 CD14+ monocytes were isolated from 30 ml of whole blood. Monocyte purity exceeded 97% with < 1% contaminating T or B cells as determined by flow cytometry (data not shown).

In vitro stimulation of monocytes

Monocytes for *in vitro* experiments were isolated from HIV-1 seronegative healthy donors (Blood Centers of the Pacific, San Francisco, CA). In brief, cells were flushed from leukoreduction filters with 40 ml PBS (Ca^{2+} and Mg^{2+} -free) and monocytes (5×10^6 cells/filter) were CD14 positively selected by immunomagnetic separation. CD14+ cells were treated with 50 U/ml IFN- α 2a (PBL Biomedical Laboratories, Piscataway, NJ), 100 U/ml IFN- γ (R&D Systems, Minneapolis, MN) or 1 ng/ml LPS obtained from *E. coli* 0127:B8 (Sigma, St Louis, MO) in RPMI-1640 supplemented with 10% FBS, 1.0 μ g/ml gentamicin and 2 mM L-glutamine at 37°C and 5% CO_2 for 48 h in Costar Ultra Low Attachment plates (Corning, Lowell, MA).

RNA isolation and monocyte gene expression analysis

Total RNA was isolated from monocytes using the RNeasy Micro Kit (Qiagen, Valencia, CA). RNA integrity was evaluated on the Agilent Bioanalyzer 2100 using a RNA 6000 Pico LabChip (Agilent Technologies, Palo Alto, CA) and all samples had a RIN value exceeding 8 [19]. Complementary RNA (cRNA) was synthesized and labeled with biotin using iExpress iAmplify kit (Applied Microarrays, Tempe, AZ). cRNA was hybridized to Codelink Whole Human Genome Bioarrays (55K probes, Applied Microarrays). The hybridization signal was acquired on an Axon GenePix 4000B scanner (Molecular Devices, Sunnyvale, CA) and image analysis and data extraction were performed by CodeLink Expression Software Kit v4.1 (GE Healthcare). Gene expression data for the HIV-1 infected subjects and HIV-1 seronegative subjects are available at NCBI GEO database, accession number GSE18464.

Microarray data were normalized with loess normalization using R [20] and Bioconductor package [21]. Determination of differential gene expression significance and multiple testing correction / false discovery rate adjustments [22] were performed using GeneSpring GX 7.3 software package (Agilent). Gene ontology analysis (<http://www.geneontology.org/index.shtml>) was conducted using the GeneSpring GX software package. Correlations between *in vitro* and *in vivo* gene expression patterns were evaluated using multiple non-parametric tests within the GraphPad Prism v5.0b Statistical Analysis Software Package.

Plasma LPS and sCD14

LPS levels in subject plasma were quantified using the Pyrogene Recombinant Factor C Endotoxin Detection System (Lonza, Walkersville, MD) according to manufacturer's protocol. Briefly, plasma samples were diluted 1:50 in pyrogen-free water before incubation with rFC working reagent. Fluorescence was measured at time zero and again at 1 h with a SpectroMax microplate reader and endotoxin concentrations were determined using a

standard curve. Soluble CD14 levels in plasma samples were quantified by ELISA with the Quantikine Human sCD14 Immunoassay (R&D Systems, Minneapolis, MD) according to the manufacturer's protocol. Samples were assayed in triplicate.

Results

High HIV-1 viral load induces an activated monocyte phenotype

HIV-1 infection radically alters the monocyte phenotype, which is reflected in an HIV-1-induced gene expression profile. To characterize the monocyte transcriptome during HIV-1 infection, global gene expression analysis using high-density cDNA microarrays was performed on cells isolated from 55 HIV-1-seropositive subjects and 10 HIV-1 seronegative controls. HIV-1-seropositive subjects were subdivided into two groups based on a clinical definition of viral load status with 22 subjects with LVL (<10,000 RNA copies/ml) and 22 subjects with HVL (>10,000 RNA copies/ml). Monocyte gene expression in the LVL group compared to controls, identified one gene that met the threshold of differential expression (DE) (≥ 2 -fold up- or down-regulated with a $P \leq 0.05$ as determined by student's t-test following correction for multiple comparison). The number of DE genes increased to 139 for HVL vs. control and there were 99 DE genes for HVL vs. LVL. (Gene list with fold change for HVL vs LVL, HVL vs controls and LVL vs controls in supplemental data 1).

Chronic immune activation correlates with HIV-1 viremia

While the interplay between HIV-1 and the immune system has been extensively studied (review [1]), dysregulation of circulating monocytes has received little attention. To characterize monocyte dysfunction, we developed a chronic immune activation (CIA) index based on a limited number of genes elevated in HIV-1-infected subjects. The index is comprised of 19 genes that are related to immune activation and are up-regulated 3 to 54-fold in HIV-1-infected subjects including those with undetectable viral loads, which were arbitrarily assigned 50 RNA copies/ml (Fig. 1A). Subclassification of gene function includes signal transduction, inflammatory response and chemotaxis (Fig. 1B). To generate a CIA value for each subject, the mean intensity of all 19 genes were taken for each sample and

calculated as follows:
$$CIA = \frac{\sum_{i=1}^n Intensity_i}{n}$$
 where intensity equals corrected and normalized signal intensity for each gene and n equals the 19 selected genes. For each subject, the CIA value and corresponding viral load were plotted, and linear regression analysis showed a Pearson's coefficient of $R^2 = 0.715$ with $P < 0.0001$ (Fig. 1C). Correlation between CIA and log viral load indicates that the CIA index can be used to define monocyte activation in HIV-1-infected subjects (Fig. 1C).

IFN- α drives CD14⁺ monocyte gene expression in HIV-1 high viral load subjects

Of numerous secreted factors that are present in the plasma and characterized as inducers of monocyte gene expression, IFN- α , IFN- γ and LPS have been shown to be elevated in HIV-1-infected subjects. To determine which of these factors was principally responsible for driving monocyte gene expression in the periphery, we treated freshly isolated CD14⁺ monocytes obtained from three healthy HIV-1-seronegative individuals with IFN- α 2a, IFN- γ or LPS for 48 h. Monocyte RNA was analyzed with the same 55K high-density microarrays that were used for the HIV-1 patient samples. From each CD14⁺ monocyte preparation, one untreated and three treated (IFN- α 2a, IFN- γ or LPS) cultures were harvested and analyzed for gene expression. Following the same DE criteria used for patient monocytes (≥ 2 -fold up- or down-regulated with a $P \leq 0.05$ as determined by student's t-test following correction for multiple comparison), IFN- α -treated cultures had 224 DE genes, IFN- γ -treated cultures had 107 DE genes and LPS, 992 DE genes (Fig. 2A). To present these results from the

patient monocyte perspective, the heat map for control, LVL and HVL subjects illustrates the induced HVL profile and the genes in each *in vitro* culture that are similarly expressed (Fig. 2B). Of the various treatments, IFN- α produced an expression profile exhibiting the highest concordance with 49 genes that were also expressed in the patient HVL monocytes. Of the 107 DE genes in the IFN- γ -treated cultures, there were only three unique genes expressed in common with the HVL group. For LPS, only one gene was similarly regulated in the HVL group.

While the heatmap (Fig. 2A) illustrates that HVL and IFN- α create overlapping monocyte expression profiles, further analysis was required to demonstrate that IFN- α was indeed a factor driving monocyte gene expression in HVL subjects. We first sought to determine that the number of DE genes shared by both IFN- α and HVL status was statistically significant. High-density microarrays in these experiments have 54,936 probes of which 96 genes were DE by the HVL group and 224 genes were DE following IFN- α treatment. There were 49 DE genes in common between HVL and IFN- α -treated monocytes (Fig. 3A). Chi-square analysis revealed a highly significant association between the sets of DE genes in these two groups ($P < .0001$). We next investigated whether genes up-regulated in the HVL group were elevated in the same hierarchical rank order in the IFN- α -treated monocytes. Using the Spearman's rank test (a conservative nonparametric measurement of correlation), the correlation in gene expression levels between HVL and IFN- α treatment was found to be highly significant ($r=0.789$, $p < 0.0001$). Even when two of the highest expressed genes were deleted from the analysis (GE88530, GE81525), the relationship remained significant ($r=0.755$, $p < 0.0001$) (Fig. 3B).

LPS and sCD14 are elevated in plasma of HIV-1-infected subjects with HVL

The absence of an LPS-associated gene expression profile in HVL subjects was curious since monocytes treated *in vitro* with LPS exhibited a significant gene response and there are numerous reports of elevated LPS levels in the periphery during HIV-1 infection [2,16,23]. To specifically evaluate our cohort, we assayed patient plasma samples reserved from the PBMC/CD14⁺ monocyte isolations and found a significant increase in LPS concentration in both LVL and HVL compared to controls (Fig. 4A). To confirm the LPS results, we assayed plasma samples for sCD14, which has been shown to be a marker with high correlative value for LPS [4]. Similar to LPS, mean sCD14 levels increased significantly between controls and HIV-1 LVL, and between LVL and HVL (Fig. 4B). These two assays clearly demonstrate elevated LPS in the periphery and similar to IFN- α -induced gene expression, increased with viral load.

To resolve the apparent inconsistency between elevated LPS levels in the periphery and absence of any LPS-induced gene expression in HVL subjects, genes known to be up-regulated in the LPS-treated monocytes were specifically queried in the HVL data set. Four genes, CXCL3, IL-10, IL-6 and IL-1 β were selected because they were significantly elevated in the LPS-treated monocytes and have been described in the literature as regulated by LPS [3,24] (Table). In all three group comparisons, HVL/C, HVL/LVL and LVL/C, there was no significant increase in expression, not even a trend. These results suggest that circulating monocytes are desensitized to LPS found in the plasma of HIV-1-infected subjects.

Discussion

Our data provide detailed information on the effect of chronic HIV-1 infection on the gene expression profile of circulating CD14⁺ monocytes. Global gene expression of peripheral monocytes was determined immediately following isolation from infected and control subjects thus providing accurate representation of the status of peripheral cells that play a

critical role in innate immunity. Gene expression analysis revealed that maintaining a low viral load through effective HAART treatment resulted in a monocyte expression profile similar to that of HIV-1-seronegative controls. In contrast, HIV-1-infected subjects with high viral loads (> 10,000 RNA copies/ml) had an average of 139 DE genes. The predominant characteristic of the monocyte profile was the number of type I IFN-regulated genes with expression intensities that correlated with viral load. While our findings confirm the report by Tilton et al. of an elevated type I interferon monocyte gene expression profile in subjects with HVL [9], we provide new evidence that IFN- α is the primary driver of gene expression during viremia. Previously, we identified a number of genes elevated in CD14+ monocytes from HIV-1-infected subjects and found that genes up-regulated in subjects with high viral loads were characteristic of an activated macrophage phenotype [18].

Peripheral monocytes in HIV-1-infected subjects with HVL have a gene expression pattern that is characteristic of chronic immune activation. We have defined the gene expression profile as the CIA index, which reflects the chronic activation state of monocytes in HIV-1-infected individuals with HVL. In both humans and non-human primates, elevated IFN- α was associated with acute HIV-1 and SIV infection, respectively [7] [8] and in the chronic HIV-1 infection phase, serum IFN- α levels correlated with disease progression and diminished benefit from anti-retroviral therapy [25]. HIV-1 stimulates plasmacytoid dendritic cell (pDC) production of IFN- α through activation of TLR-7 and -9, which recognize single stranded viral RNA and unmethylated CpG-rich DNA, respectively [26,27]. pDC are the primary source of IFN- α in HIV-1 infection [28], producing up to 1000-fold more IFN than other cell types [29]. While high viral load generally equates with elevated expression of IFN- α responsive genes, there are subjects with high viral loads that exhibit below average expression of IFN responsive genes. This may be due to a loss of IFN- α -producing pDC through either cell death [30], migration to lymph nodes [31] or alternatively through decreased monocyte sensitivity to IFN- α stimulation [32]. Despite these mechanisms, which in combination would reduce the number of pDC in the periphery and dampen monocyte response to IFN- α , the majority of HVL subjects retain an IFN- α -induced phenotype. It will be important to track monocyte activation and pDC populations longitudinally to understand how these variables affect disease progression.

While the IFN- γ -treated cultures share a considerable number of DE genes in common with HVL, it is important to note that genes in this treatment group were also DE genes in the IFN- α treatment group. Since type I and II IFNs share intracellular signaling pathways and therefore activate a number of genes in common, designating DE genes in the IFN- γ column as exclusively IFN- γ -induced genes is questionable. There were only three unique IFN- γ -induced genes expressed in common with the HVL group. Therefore, without additional evidence, it is reasonable to assume the majority of DE genes in the HVL group were induced by IFN- α and not IFN- γ . This assessment is supported by a study that tracked plasma IFN- α and IFN- γ levels in asymptomatic and symptomatic HIV-1-infected subjects following HAART [25]. Subjects with symptomatic HIV-1 infection had the highest IFN- α levels, while asymptomatic subjects had significantly lower levels albeit above controls. Furthermore, suppression of virus replication by HAART decreased IFN- α levels while non-responders to HAART retained elevated IFN- α levels. In these same subjects, IFN- γ levels remained low regardless of HAART treatment and were not different from controls [25].

Lipopolysaccharide entering the periphery through a dysfunctional GI tract damaged by T cell decimation has been proposed as the principal factor in chronic immune activation [2,33]. Bacterial LPS is a powerful activator of monocytes and other immune active cells that express CD14 and TLR-4. Our *in vitro* results clearly demonstrate the capacity of CD14+ monocytes to respond to LPS with over 992 DE genes following a single dose of ultra-pure, *E. coli*-derived LPS. However, there was no LPS gene expression profile in

monocytes isolated from subjects with HVL. In fact, only 1 LPS-related gene was differentially regulated in common with the HVL profile. Lipopolysaccharide in the periphery was confirmed by detection of both LPS and a correlative marker, sCD14, where both were significantly elevated in subjects with HVL. Considering the possibility that subject monocytes responded to LPS but did not exceed the threshold set for differential gene expression, we examined a number of genes that are characterized as LPS responsive genes that were significantly elevated in the LPS-treated monocytes. None of these genes showed an increase in expression despite multi-fold increases in the *in vitro*-treated monocytes. A possible interpretation is that long-term exposure to sub-optimal doses of LPS has produced a deactivated state rendering the monocytes non-responsive to bacterial products [34]. Our findings indicate that CD14+ monocytes, which would be expected to respond vigorously to systemic LPS, are at minimum desensitized and are not contributing to the immune activation observed in HIV-1 disease. In fact, our observations are consistent with other reports indicating that viral load and not LPS is responsible for chronic immune activation [4,23]. Future work should be directed at identifying LPS-responsive cells *in vivo* that may be responsible for chronic immune activation.

Our data suggests that IFN- α in plasma of HVL subjects has a profound impact on monocytes, which is significant because it alters monocyte function and possibly dysregulates normal monocyte maturation into macrophages and DC. We have studied one of the HVL genes, sialoadhesin (Sn), showing its expression correlated with HIV-1 viral load and was inducible by both IFN- α and - γ *in vitro* [35]. In HIV-1 disease, Sn is induced to high levels shortly after infection and remains elevated during disease progression [36]. Sialoadhesin is a large 190 kDa transmembrane protein thought to mediate cell-cell interaction through binding of Neu5Ac sialic acids on adjacent cells [37] and we have demonstrated the capacity of Sn to effect HIV-1 trans infection of susceptible cells [35]. Up-regulating this single gene has multiple consequences ranging from enhanced cell-cell interactions to increased infectivity with possible implications for immune activation. Considering the potential for an expanded role of IFN- α -induced monocytes in HIV-1 disease progression, accurate characterization of the IFN- α -induced disease phenotype will prove critical in assessing the role of these dynamic cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Douek DC, Roederer M, Koup RA. Emerging concepts in the immunopathogenesis of AIDS. *Annu Rev Med* 2009;60:471–484. [PubMed: 18947296]
2. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* 2006;12:1365–1371. [PubMed: 17115046]
3. Guha M, Mackman N. LPS induction of gene expression in human monocytes. *Cell Signal* 2001;13:85–94. [PubMed: 11257452]
4. Papasavvas E, Pistilli M, Reynolds G, Bucki R, Azzoni L, Chehimi J, et al. Delayed loss of control of plasma lipopolysaccharide levels after therapy interruption in chronically HIV-1-infected patients. *Aids*. 2008

5. Suffredini AF, Hochstein HD, McMahon FG. Dose-related inflammatory effects of intravenous endotoxin in humans: evaluation of a new clinical lot of *Escherichia coli* O:113 endotoxin. *J Infect Dis* 1999;179:1278–1282. [PubMed: 10191237]
6. Herbeuval JP, Shearer GM. HIV-1 immunopathogenesis: how good interferon turns bad. *Clin Immunol* 2007;123:121–128. [PubMed: 17112786]
7. von Sydow M, Sonnerborg A, Gaines H, Strannegard O. Interferon-alpha and tumor necrosis factor-alpha in serum of patients in various stages of HIV-1 infection. *AIDS Res Hum Retroviruses* 1991;7:375–380. [PubMed: 1906289]
8. Khatissian E, Tovey MG, Cumont MC, Monceaux V, Lebon P, Montagnier L, et al. The relationship between the interferon alpha response and viral burden in primary SIV infection. *AIDS Res Hum Retroviruses* 1996;12:1273–1278. [PubMed: 8870849]
9. Tilton JC, Johnson AJ, Luskin MR, Manion MM, Yang J, Adelsberger JW, et al. Diminished production of monocyte proinflammatory cytokines during human immunodeficiency virus viremia is mediated by type I interferons. *J Virol* 2006;80:11486–11497. [PubMed: 17005663]
10. Lane HC, Davey V, Kovacs JA, Feinberg J, Metcalf JA, Herpin B, et al. Interferon-alpha in patients with asymptomatic human immunodeficiency virus (HIV) infection. A randomized, placebo-controlled trial. *Ann Intern Med* 1990;112:805–811. [PubMed: 1971503]
11. Rodriguez B, Lederman MM, Jiang W, Bazdar DA, Garate K, Harding CV, et al. Interferon-alpha differentially rescues CD4 and CD8 T cells from apoptosis in HIV infection. *Aids* 2006;20:1379–1389. [PubMed: 16791012]
12. Sedaghat AR, German J, Teslovich TM, Cofrancesco J Jr, Jie CC, Talbot CC Jr, et al. Chronic CD4+ T-cell activation and depletion in human immunodeficiency virus type 1 infection: type I interferon-mediated disruption of T-cell dynamics. *J Virol* 2008;82:1870–1883. [PubMed: 18077723]
13. Francis ML, Meltzer MS, Gendelman HE. Interferons in the persistence, pathogenesis, and treatment of HIV infection. *AIDS Res Hum Retroviruses* 1992;8:199–207. [PubMed: 1371692]
14. Zagury D, Lecoq H, Gervi I, Le Buanec H, Zagury JF, Bizzini B, et al. Anti-IFN alpha immunization raises the IFN alpha-neutralizing capacity of serum--an adjuvant to antiretroviral tritherapy. *Biomed Pharmacother* 1999;53:90–92. [PubMed: 10337463]
15. Gringeri A, Musicco M, Hermans P, Bentwich Z, Cusini M, Bergamasco A, et al. Active anti-interferon-alpha immunization: a European-Israeli, randomized, double-blind, placebo-controlled clinical trial in 242 HIV-1--infected patients (the EURIS study). *J Acquir Immune Defic Syndr Hum Retrovirol* 1999;20:358–370. [PubMed: 10096580]
16. Ancuta P, Kamat A, Kunstman KJ, Kim EY, Autissier P, Wurcel A, et al. Microbial translocation is associated with increased monocyte activation and dementia in AIDS patients. *PLoS ONE* 2008;3:e2516. [PubMed: 18575590]
17. Sonza S, Maerz A, Deacon N, Meanger J, Mills J, Crowe S. Human immunodeficiency virus type 1 replication is blocked prior to reverse transcription and integration in freshly isolated peripheral blood monocytes. *J Virol* 1996;70:3863–3869. [PubMed: 8648722]
18. Pulliam L, Sun B, Rempel H. Invasive chronic inflammatory monocyte phenotype in subjects with high HIV-1 viral load. *J Neuroimmunol* 2004;157:93–98. [PubMed: 15579285]
19. Schroeder A, Mueller O, Stocker S, Salowsky R, Leiber M, Gassmann M, et al. The RIN: an RNA integrity number for assigning integrity values to RNA measurements. *BMC Mol Biol* 2006;7:3. [PubMed: 16448564]
20. Ihaka R, Gentleman R. R: A Language for Data Analysis and Graphics. *Journal of Computational and Graphical Statistics* 1996;5:299–314.
21. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, et al. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol* 2004;5:R80. [PubMed: 15461798]
22. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society, Series B (Methodological)* 1995;57:125–133.

23. Gregson JN, Steel A, Bower M, Gazzard BG, Gotch FM, Goodier MR. Elevated plasma lipopolysaccharide is not sufficient to drive natural killer cell activation in HIV-1-infected individuals. *Aids* 2009;23:29–34. [PubMed: 19050383]
24. Goodall JC, Ellis L, Yeo GS, Gaston JS. Does HLA-B27 influence the monocyte inflammatory response to lipopolysaccharide? *Rheumatology (Oxford)* 2007;46:232–237. [PubMed: 16877465]
25. Stylianou E, Aukrust P, Bendtzen K, Muller F, Froland SS. Interferons and interferon (IFN)-inducible protein 10 during highly active anti-retroviral therapy (HAART)-possible immunosuppressive role of IFN-alpha in HIV infection. *Clin Exp Immunol* 2000;119:479–485. [PubMed: 10691920]
26. Colonna M, Trinchieri G, Liu YJ. Plasmacytoid dendritic cells in immunity. *Nat Immunol* 2004;5:1219–1226. [PubMed: 15549123]
27. Zhang Z, Wang FS. Plasmacytoid dendritic cells act as the most competent cell type in linking antiviral innate and adaptive immune responses. *Cell Mol Immunol* 2005;2:411–417. [PubMed: 16426490]
28. Lehmann C, Harper JM, Taubert D, Hartmann P, Fatkenheuer G, Jung N, et al. Increased interferon alpha expression in circulating plasmacytoid dendritic cells of HIV-1-infected patients. *J Acquir Immune Defic Syndr* 2008;48:522–530. [PubMed: 18645522]
29. Kadowaki N, Antonenko S, Lau JY, Liu YJ. Natural interferon alpha/beta-producing cells link innate and adaptive immunity. *J Exp Med* 2000;192:219–226. [PubMed: 10899908]
30. Soumelis V, Scott I, Gheys F, Bouhour D, Cozon G, Cotte L, et al. Depletion of circulating natural type 1 interferon-producing cells in HIV-infected AIDS patients. *Blood* 2001;98:906–912. [PubMed: 11493432]
31. Malleret B, Maneglier B, Karlsson I, Lebon P, Nascimbeni M, Perie L, et al. Primary infection with simian immunodeficiency virus: plasmacytoid dendritic cell homing to lymph nodes, type I interferon, and immune suppression. *Blood* 2008;112:4598–4608. [PubMed: 18787223]
32. Hardy GA, Sieg SF, Rodriguez B, Jiang W, Asaad R, Lederman MM, et al. Desensitization to type I interferon in HIV-1 infection correlates with markers of immune activation and disease progression. *Blood* 2009;113:5497–5505. [PubMed: 19299650]
33. Brechley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ, et al. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J Exp Med* 2004;200:749–759. [PubMed: 15365096]
34. Fujihara M, Muroi M, Tanamoto K, Suzuki T, Azuma H, Ikeda H. Molecular mechanisms of macrophage activation and deactivation by lipopolysaccharide: roles of the receptor complex. *Pharmacol Ther* 2003;100:171–194. [PubMed: 14609719]
35. Rempel H, Calosing C, Sun B, Pulliam L. Sialoadhesin expressed on IFN-induced monocytes binds HIV-1 and enhances infectivity. *PLoS ONE* 2008;3:e1967. [PubMed: 18414664]
36. van der Kuyl AC, van den Burg R, Zorgdrager F, Groot F, Berkhout B, Cornelissen M. Sialoadhesin (CD169) Expression in CD14+ Cells Is Upregulated Early after HIV-1 Infection and Increases during Disease Progression. *PLoS ONE* 2007;2:e257. [PubMed: 17330143]
37. Crocker PR, Clark EA, Filbin M, Gordon S, Jones Y, Kehrl JH, et al. Siglecs: a family of sialic-acid binding lectins. *Glycobiology* 1998;8:v. [PubMed: 9498912]

Gene	Fold change
IFI27	54.5
SN	12.0
LGALS3BP	7.7
SERPING1	7.2
IFI1T1	6.2
IFI1T1M1	6.0
DEFB1	5.3
MX1	5.3
OASL	5.1
CXCL11	5.0
CFB	4.7
CCL8	4.6
IFI1T3	4.5
CCL2	4.4
IFI1T2	4.2
CXCL10	3.8
C1QC	3.7
GBP1	3.2
STAP1	3.0

Fig. 1a

Fig. 1b

Fig. 1c

Fig. 1. Chronic immune activation index (CIA)

(a) List of genes and their fold change from the microarray analysis of DE genes with a ≥ 3 fold elevated expression in HVL vs control. (b) Associated Gene Ontology categories for genes in the CIA index. (c) Correlation of CIA index and viral load for subjects with detectable viral loads. Data are shown with regression line, correlation coefficient and significance.

Fig. 2a

Fig. 2b

Fig. 2. Comparison of monocyte gene expression from HIV subjects and *in vitro* treatments
 (A) Heat maps of CD14+ monocytes treated *in vitro* with IFN- α (50 U/ml), IFN- γ (100 U/ml), LPS (1 ng/ml) or untreated (NT) illustrating the monocyte response to these inducers.

Far right column, DE genes from HIV_{HVL} subjects that correspond to DE genes in IFN- α , IFN- γ , or LPS treatment groups. Differentially expressed genes had a minimum 2-fold change, $P < 0.05$ significance and corrected for multiple comparisons. (B) Heat maps generated from microarray analyses of monocytes isolated from control subjects ($n = 11$) HIV-infected subjects with low (LV, $n = 22$) and high (HVL, $n = 22$) viral load. CD14⁺ monocytes from HIV-seronegative individuals treated *in vitro* with IFN- α , IFN- γ or LPS for 48 h. Differentially expressed (DE) genes in the HVL group that are also DE genes in the *in vitro* treated monocytes are indicated on the right. As described above, DE genes had a minimum 2-fold change, $p < 0.05$ significance and corrected for multiple comparisons. Expression bar indicates relative expression levels.

Fig. 3a

Fig. 3b

Fig. 3. HVL and IFN- α -induced monocyte gene expression profiles

(a) Venn diagram illustrating the overlapping pattern of gene expression. Total number of microarray probes (54567) that were not differentially expressed (DE) by either HVL or IFN- α treatment; number of unique DE genes to HIV-infected subjects with HVL (pink, 96); number of DE genes unique to monocytes treated with IFN- α 2a (50 U/ml) (green, 224); DE genes expressed in both HVL and IFN- α -treated monocytes (49). (b) Pairwise comparison of HVL and IFN- α -induced gene expression using Spearman's nonparametric measurement of correlation. Data are shown with regression lines, correlation coefficients and significance.

Fig. 4. Plasma levels of LPS and sCD14 in HIV-infected subjects

Plasma samples were reserved from PBMC enrichment and assayed for LPS using the Pyrogen protocol and soluble (s)CD14 by ELISA. Concentration of (a) LPS and (b) sCD14 were determined for controls (n=10), low viral load (LVL, n=21) and high viral load (HVL, n=22) subjects. Statistically significance between groups was determined by Student's t-test and *P* values are shown.

Table 1Expression of selected genes induced by LPS *in vitro* and in HIV-1 subject monocytes

Gene	Fold change			
	LPS/NT ¹	HVL/C ²	HVL/LVL ³	LVL/C
CXCL3	60	1.1	1.2	1.0
IL10	61	0.8	1.4	0.6
IL6	45	1.4	1.2	1.1
IL-1 β	6	1.1	1.5	0.7

¹ LPS-treated monocytes vs not treated (NT)² HVL/C – high viral load / control subjects³ HVL/LVL – high viral load / low viral load subjects