Desensitization to type I interferon in HIV-1 infection correlates with markers of immune activation and disease progression

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Type I interferon (IFNα/β) plays a complex role in HIV-1 infection and has been proposed alternately to have roles in either disease protection or progression. Although IFNα/β plays crucial roles in regulating monocytes and dendritic cells, responsiveness of these cells to IFNα/β in HIV-1 infection is poorly understood. We report significant defects in IFNα/β receptor (IFNα/βR) expression, IFNα signaling, and IFNα-induced gene expression in monocytes from HIV-1–infected subjects. IFNα/βR expression correlated directly with CD4+ T-cell count and inversely with HIV-1 RNA level and expression of CD38 by memory (CD45RO+) CD8+ T cells, a measure of pathologic immune activation in HIV-1 infection associated with disease progression. In addition, monocytes from HIV-1–infected persons showed diminished responses to IFNα, including decreased induction of phosphorylated STAT1 and the classical interferon-stimulated gene produces MxA and OAS. These IFNα responses were decreased regardless of IFNα/βR expression, suggesting that regulation of intracellular signaling may contribute to unresponsiveness to IFNα/β in HIV-1 disease. Defective monocyte responses to IFNα/β may play an important role in the pathogenesis of HIV-1 infection, and decreased IFNα/βR expression may serve as a novel marker of disease progression. (Blood. 2009;113: 5497-5505)

Introduction

HIV-1 infection disrupts numerous elements of the innate immune system.[1,2] At the interface between innate and acquired immune responses, antigen-presenting cells (APCs) such as dendritic cells (DCs) and monocytes/macrophages recognize distinct microbial structures through Toll-like receptors (TLRs) and other pattern recognition receptors.[3] Signaling through these receptors induces expression of cytokines, such as type I IFN (IFNα/β), that promote innate immunity and APC maturation.[4,5] Our previous studies showed that TLR9 agonist stimulation of unfractionated peripheral blood mononuclear cells (PBMCs) generates monocyte responses that are defective in HIV-1 infection[6]; these studies implicated both reduced TLR induction of IFNα/β and reduced monocyte responsiveness to IFNα/β as potential mechanisms in HIV-1 infection. Despite its potential significance to HIV-1 pathogenesis, knowledge of IFNα/β signaling and its regulation in HIV-1 disease remains limited.

IFNα/β comprises 13 different functional isoforms of IFNα and 1 IFNβ, all of which signal through the same IFNα/β receptor (IFNα/βR), a heterodimer composed of IFNAR1 and IFNAR2.[7] IFNα/βR signaling activates tyrosine kinase 2 (TYK2) and Janus kinase 1 (JAK1), which in turn phosphorylate signal transducer and activator of transcription 1 (STAT1) and STAT2.[8] Phosphorylated STAT1 and STAT2 heterodimerize and associate with interferon regulatory factor-9 (IRF-9) to form interferon-stimulated gene factor-3 (ISGF-3). ISGF-3 binds to interferon-stimulated response elements in the promoters of hundreds of interferon-stimulated genes (ISGs), including the genes for the myxovirus resistance protein A (MxA) and 2′, 5′ oligoadenylate synthase-3 (OAS).[8] IFNα/β is produced by a variety of cell types, particularly plasmacytoid DCs (pDCs),[9,10] which produce up to 1000-fold more IFNα/β than other cell types.[11] IFNα/β has pleiotropic effects on many cell types, including direct antiviral effects, differential promotion of cell survival and apoptosis, inflammatory effects and enhancement of differentiation, and maturation of blood myeloid DCs (mDCs) and monocytes into potent T-cell stimulators.[12,13]

Despite its well-characterized antiviral activity, the role of IFNα/β in HIV-1 infection is controversial, with conflicting observations suggesting protective versus pathologic roles. Administration of recombinant human IFNα may have beneficial effects during the asymptomatic phase of HIV-1 infection, stabilizing CD4 decline and reducing the incidence of AIDS-defining events,[14] although these effects are not observed in more advanced disease.[15] Transiently high levels of endogenous serum IFNα have been described in primary HIV-1 infection[16] and acute simian immunodeficiency virus infection in macaques.[17] During the asymptomatic phase of chronic HIV-1 infection, elevated serum IFNα levels are found at increasing frequency with advancing disease progression, reaching high levels in late-stage HIV-1 infection[16] and correlating with poor outcomes in response to antiretroviral therapy.[18] IFNα/β may protect T cells from spontaneous apoptosis, but this effect is reduced in HIV-1 disease.[19] Other studies suggest that IFNα/β may contribute to bystander apoptosis of uninfected CD4+ T cells[20] and that depletion of CD4+ T cells in HIV-1 infection may be mediated by IFNα/β-induced activation.[21] Alternatively, the late increase in IFNα/β may be a result, rather than a cause, of disease progression and may reflect increasing pathologic immune activation, driven by HIV-1 itself, opportunistic pathogens, or other microbial stimuli.[22] We propose that responses to IFNα/β may be desensitized in late

HIV-1 infection, potentially explaining the lack of efficacy of recombinant human IFNα therapy at that stage.

The studies presented here show a significant loss of monocyte responsiveness to IFNα/β in HIV-1 infection. We observed defects at multiple levels, including decreased expression of IFNα/βR, loss of IFNα/β signaling through phosphorylated STAT1, and loss of induction of ISGs. Moreover, these defects correlated significantly with markers of disease progression in HIV-1 infection, including decreased CD4+ T-cell count and induction of CD38 on memory (CD45RO+) CD8+ T cells (expression of this activation marker on CD8+ T cells is an important predictor of HIV-1 disease progression).[23-25] Diminished responsiveness to IFNα/β may decrease responses of HIV-1–infected persons to immunotherapeutic agents or to vaccine adjuvants that act through induction of IFNα/β production, eg, CpG DNA or imiquimod, and could account for the inconsistent effects observed in clinical trials of IFNα/β for treatment of HIV-1 infection. Defects in IFNα/β responsiveness may represent an important pathologic mechanism in HIV-1 disease, leading to failure of innate immunity and impairment of acquired immune responses. Moreover, loss of IFN-α/βR expression by monocytes correlates with markers of disease progression and could potentially serve as a novel marker to help assess or predict disease progression in HIV-1 infection.

Methods

Study subjects and cell samples

Peripheral blood was obtained from HIV-1–infected and uninfected subjects in this institutional review board–approved cross-sectional study at Case Western Reserve University. Informed consent was obtained from all subjects in accordance with the Declaration of Helsinki. HIV-1–infected participants were not receiving and had not received antiretroviral therapy or any immunotherapy in the past 2 years. HIV-1–infected and uninfected subjects donated 60 mL blood on one occasion each (into lithium heparin tubes). Plasma was separated from each sample and stored at −80°C.

Peripheral blood mononuclear cells were isolated by centrifugation with endotoxin-free Ficoll-Paque (GE Healthcare Bio-Sciences, Little Chalfont, United Kingdom). PBMCs were analyzed by flow cytometry or were used to prepare monocytes by negative selection at 4°C with the Monocyte Isolation Kit II (Miltenyi Biotec, Auburn, CA). Monocyte purity (by flow cytometry with CD14 staining) was greater than 90%.

Flow cytometry

PBMCs were stained on ice with the following murine anti–human monoclonal antibodies (from Becton Dickinson, Franklin Lakes, NJ, if not specified otherwise): anti–CD14-PE (Miltenyi Biotec), anti–CD3-FITC, anti–CD4-PerCP, anti–CD8-PerCp, Lin-1 cocktail (anti-CD3, anti-CD14, anti-CD16, anti-CD19, anti-CD20, anti-CD56)–FITC, anti–HLA-DR-PerCP, anti–CD11c-PE, anti–CD3-APC, anti–CD45RO-FITC, and anti–CD38-PE. Biotin-conjugated polyclonal goat anti-IFNα/βR (specific for the IFNAR1 chain) and control normal goat IgG (R&D Systems, Minneapolis, MN) were used with streptavidin-conjugated APC. For analysis of DCs, HLA-DR+, Lin-1− cells were gated according to their expression of CD11c. CD11c+ cells were considered to be mDCs; CD11c− cells were considered to be pDCs (this exclusionary gating definition may have included a small number of non-pDCs). Stained cells were washed in PBS/0.05% sodium azide, fixed in 1% formaldehyde, and analyzed with a 4-color FACSCalibur flow cytometer and CellQuest software (Becton Dickinson) with an acquisition threshold of 20 000 gated events.

To detect phosphorylated STAT1, 10⁶ PBMCs were preincubated at 4°C for 30 minutes with anti–CD14-PE mAb (Miltenyi Biotec), washed, resuspended in 1 mL RPMI with 10% FCS (Hyclone, Logan, UT), and cultured for 15 minutes with or without IFNα2a (PBL Biomedical Laboratories, Piscataway, NJ) at 1000, 3000, or 10 000 U/mL. Cells were washed, fixed with Becton Dickinson cytofix buffer, permeabilized with Becton Dickinson Phosflow Perm Buffer III, and stained with Alexa-488–conjugated murine anti–human phosphorylated STAT1 mAb (BD PharMingen, San Diego, CA) or isotype control MOPC-173 mAb (BD PharMingen) for 30 minutes at room temperature.

Real-time polymerase chain reaction

Monocytes were resuspended in MACS (magnetic cell sorting) Rinsing Solution (Miltenyi Biotec) supplemented with 10% FCS. Equal aliquots (1-2 × 10⁶ cells) were placed immediately into RLT lysis buffer (QIAGEN, Valencia, CA) and stored at −80°C (ex vivo sample) or cultured in 24-well plates for 18 hours at 37°C in RPMI with 2 mM L-glutamine, 10 mM penicillin and streptomycin, and 5% human male AB serum (Gemini Bio-Products, West Sacramento, CA) with or without 1000 IU/mL IFNα2a (PBL Biomedical Laboratories). Supernatants were removed, cells were lysed in situ with RLT lysis buffer, and lysates were stored at −80°C. Lysates were passed through QIAshredder columns (QIAGEN), and mRNA was extracted after on-column DNase digestion with the use of the RNeasy plus kit (QIAGEN) and stored in RNase-free sterile water at −80°C.

Concentration of mRNA was determined by optical density; cDNA was reverse transcribed from mRNA using oligo(dT) primer–based Superscript II First-Strand Synthesis kit (Invitrogen, Carlsbad, CA) and quantified by real-time quantitative reverse transcription–polymerase chain reaction (qRT-PCR) in triplicate with an iCycler (Bio-Rad, Hercules, CA) with the use of SYBR green detection master mix (Abgene Limited, Epsom, United Kingdom). Absolute quantities of mRNA product were determined from a standard curve of serial dilutions of known quantities of each specific amplicon. Results were normalized to GAPDH. Primer pairs were as follows: GAPDH (sense, 5′-GACCTGACCTGCCGTCTA-3′; antisense, 5′GTTGCTGTAGCCAAATTCGTT-3′), MxA (sense, 5′-AGAAGGAGCT-GGAAGAAG-3′; antisense, 5′-CTGGAGCATGAAGAACTG-3′), OAS (sense, 5′-GACCACTGTAGCCGAATCAG-3′; antisense, 5′-TGGCAC-CCATTCAATCAT-3′), and IFNAR2 (sense, 5′-AGTCAGAGGGAATTGT-TAAGAAGCA-3′; antisense, 5′-TTTGGAATTAACTTGTCAATGATAT-AGGTG-3′). The IFNAR2 amplicon was representative of all 3 known variants of IFNAR2. Published primers[26] were used for detection of IFNAR1 (sense, 5′-CCCAGTGTGTCTTTCCTCAAA-3′; antisense, 5′-AAGACTGGAGGAAGTAGGAAAGC-3′).

IFNα enzyme-linked immunoabsorbent assay

Plasma was separated from blood taken into EDTA anticoagulant. IFNα was detected by an enzyme-linked immunoabsorbent assay (ELISA; PBL Biomedical Laboratories) that detects 12 human IFNα types (detection limit of 12.5 pg/mL). IFNα standards and neat plasma samples were incubated in precoated 96-well ELISA plates for 1 hour (all ELISA procedures were at room temperature). IFNα was detected with a biotinylated anti-IFNα antibody and streptavidin–horseradish peroxidase with tetramethyl-benzidine substrate. Optical density was measured at 450 nm with a Bio-Rad model 680 microplate reader.

Statistical analysis

We used conventional measures of central location and dispersion to describe the data. Pairs of variables were compared with Mann-Whitney U test or Wilcoxon signed rank test, depending on the relations between the groups. To explore associations between pairs of continuous variables, we used correlation analysis or simple linear regression. Relationships of multiple predictors of interest with a continuous dependent variable were assessed by multiple regression with a stepwise approach to select significant covariates. Analyses were performed with SPSS, version 16.01 (SPSS Inc, Chicago, IL) and Stata MP, version 10 (Stata Corp, College Station, TX) without explicit correction for multiple comparisons. All tests were 2-sided, and P values less than or equal to .05 were considered statistically significant.

Results

Expression of IFNα/βR is decreased on monocytes of HIV-1–infected persons

Because reduced sensitivity to IFNα/β may affect HIV-1 disease pathogenesis, we investigated monocyte expression of IFNα/βR and functional responses of monocytes to IFNα in 59 viremic HIV-1–infected subjects who were not receiving antiretroviral therapy and 32 uninfected persons in a cross-sectional study. The median age of HIV-1–infected persons at time of sample collection was 45 years (interquartile range [IQR], 39-48 years), and 26% were female. The median age for uninfected persons was 38 years (IQR, 31-47 years), and 53% were female. For HIV-infected subjects, the median absolute CD4$^+$ T-cell count was 383 cells/μL (IQR, 315-544 cells/μL), and the median HIV-1 RNA level was 26 000 copies/mL (IQR, 9500-91 700 copies/mL). The same donor samples were used for multiple analyses to assess monocyte IFNα/βR expression (this section) and functional responses to exogenous IFNα (in subsequent sections), although we were not able to perform all analyses on every sample.

Flow cytometry was used to assess expression of IFNα/βR ex vivo (ie, without in vitro incubation) on monocytes from 54 HIV-1–infected subjects and 32 uninfected persons. The monocyte gate was defined by CD14 expression and side scatter characteristics. Representative histograms of IFNα/βR expression are shown for uninfected (Figure 1A) and HIV-1–infected (Figure 1B) subjects, showing a decrease in monocyte expression of IFNα/βR in HIV-1 infection. The median IFNα/βR specific mean fluorescence intensity (sMFI), ie, MFI with anti-IFNα/βR Ab minus MFI with isotype control Ab, was 87.12 (IQR, 53.98-159.44) for uninfected persons (n = 32) and 19.18 (IQR, 8.45-47.0) for HIV-1–infected subjects (n = 54; Figure 1C). The median percentage of monocytes expressing detectable IFNα/βR was 76.52% (IQR, 19.76%-94.77%) in uninfected persons and only 14.91% (IQR, 6.25%-28.72%) for HIV-1–infected subjects (data not shown). Thus, IFNα/βR expression was significantly reduced on monocytes from HIV-1–infected subjects as assessed by both sMFI (P < .001) and the percentage of monocytes expressing detectable receptor (P < .001).

Expression of IFNα/βR is decreased on DCs but not T cells in HIV-1 infection

To determine whether the loss of IFNα/βR was restricted to monocytes or affected other cell types, we assessed IFNα/βR expression ex vivo on DCs and T cells. IFNα/βR expression ex vivo on CD11c$^+$ mDCs and CD11c$^-$ pDCs (Figure 1D) was lower than on monocytes but showed a similar pattern of reduction in HIV-1–infected subjects relative to uninfected persons. The median sMFI for IFNα/βR on mDCs was 7.98 (IQR, 0-12.44) for uninfected persons (n = 14) and 1.81 (IQR, 0-5.41) for HIV-1–infected subjects (n = 33; P = .052). Furthermore, the percentage of mDCs with positive staining for IFNα/βR was significantly lower in HIV-1–infected subjects (median, 1.69% positive; IQR, 0.01%-3.64% positive) than in uninfected persons (median, 16.81% positive; IQR, 5.93%-29.48% positive; P < .001). The median sMFI for IFNα/βR on pDCs was 7.0 (IQR, 3.0-19.0) for uninfected persons (n = 16) and 2.0 (IQR, 0-9.0) for HIV-1–infected subjects (n = 32; P = .046). The percentage of pDCs with positive staining for IFNα/βR was also higher in uninfected persons (median, 5.82%
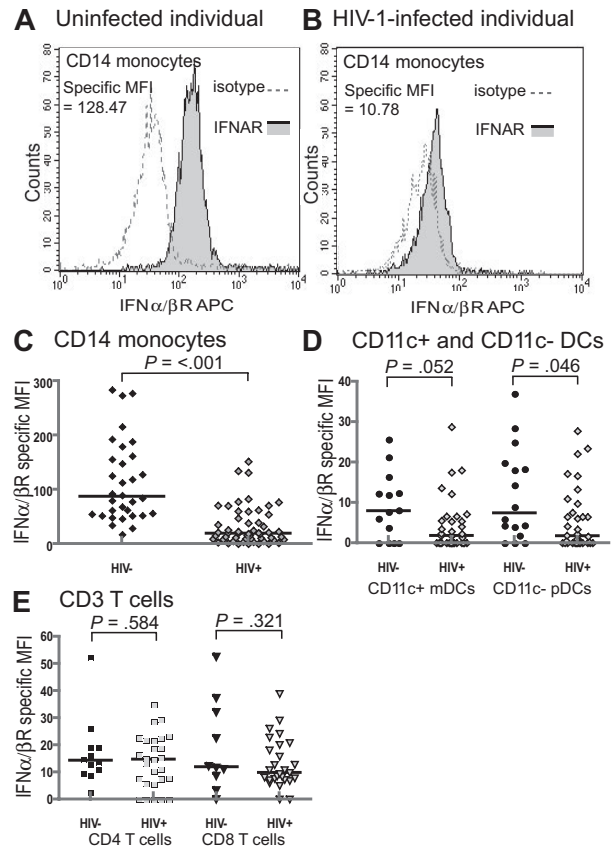
Figure 1. IFNα/βR expression is diminished on monocytes of HIV-1–infected persons. Individual flow cytometry histograms show IFNα/βR expression (solid curve) with isotype control (dashed line) on CD14-gated monocytes from uninfected (A) and HIV-1–infected (B) persons. IFNα/βR expression was significantly diminished on CD14$^+$ monocytes of HIV-1–infected persons compared with uninfected persons (P < .001; C). IFNα/βR expression was also diminished on CD11c$^+$ mDCs and CD11c$^-$ pDCs of HIV-1–infected persons compared with uninfected subjects (P = .052 and P = .046, respectively; D). DCs were defined according to the expression of CD11 on the gated HLA-DR bright, Lin1$^-$ population (see "Methods"). HIV-1 infection was not associated with significant reductions in IFNα/βR expression on CD3$^+$/CD4$^+$ or CD3$^+$/CD8$^+$ T cells (P = .584 and P = .321, respectively; E) Horizontal bars in panels C through E represent median values.

positive; IQR, 2.14%-10.89% positive) than in HIV-1–infected subjects (median, 1.5% positive; IQR, 0.03%-2.6% positive; P = .002). Thus, HIV-1 infection was associated with reduced expression of IFNα/βR on mDCs and pDCs.

IFNα/βR expression on CD4$^+$ and CD8$^+$ T cells was lower than on monocytes and was not significantly altered by HIV-1 infection (Figure 1E). The median sMFI for IFNα/βR expression on CD4$^+$ T cells was 14.4 (IQR, 10.4-19) for uninfected persons (n = 12) versus 14.7 (IQR, 5.9-21.9) for HIV-1–infected subjects (n = 27; P = .584). The percentage of CD4$^+$ T cells that expressed IFNα/βR was 15.85% (IQR, 12.24%-40.05%) for uninfected persons versus 11.16% (IQR, 3.75%-28.43%) for HIV-1–infected subjects (P = .181; data not shown). Similarly, the median sMFI on CD8$^+$ T cells was 11.94 (IQR, 8.6-32.2) for uninfected persons (n = 10) and 9.8 (IQR, 7.3-20.1) for HIV-1–infected subjects (P = .321), and the percentage of CD8$^+$ T cells that expressed IFNα/βR was 9.32% (IQR, 4.36%-46.48%) for uninfected persons versus 5.51% (IQR, 2.73%-9.39%) for HIV-1–infected subjects (P = .141; data not shown). Thus, we did not observe a statistically significant change in IFNα/βR expression by CD4$^+$ or CD8$^+$ T cells of HIV-1–infected persons. We conclude that decreased
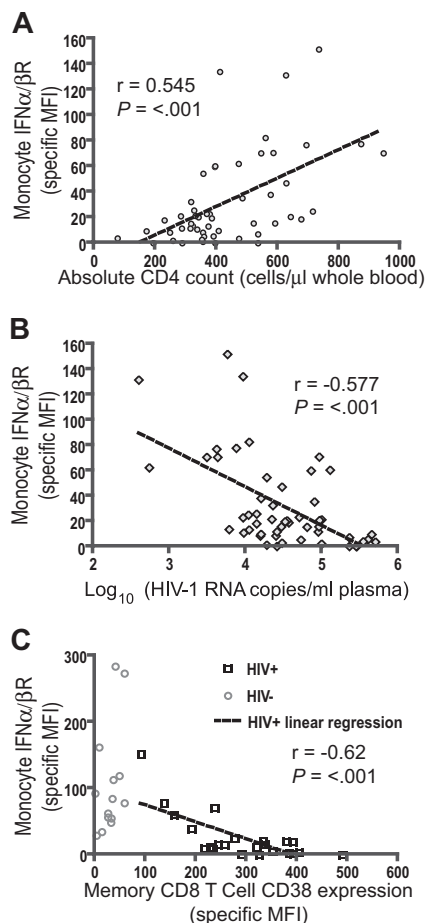
Figure 2. Loss of IFNα/βR on monocytes correlates with markers of disease progression in HIV-1 infection. Univariate linear regression analysis was used to assess relationships of disease markers with IFNα/βR expression. In HIV-1–infected persons, monocyte expression of IFNα/βR correlated with absolute CD4+ T-cell count (A; $P < .001$), correlated inversely with HIV-1 RNA level (B; $P < .001$), and correlated inversely with CD38 expression on memory (CD45RO+) CD8+ T cells (C; $P < .001$). In panel C, there was no significant correlation between monocyte IFNα/βR and CD38 expression on memory CD8 T cells in uninfected persons.

IFNα/βR expression in the setting of HIV-1 infection was detected on monocytes, mDCs, and pDCs, but was not observed on T cells.

Monocyte IFNα/βR expression correlates directly with peripheral absolute CD4+ T-cell count and inversely with plasma HIV-1 RNA levels and memory CD8+ T-cell immune activation

We investigated the potential correlation of IFNα/βR expression with clinical indicators of HIV-1 disease progression. We focused these analyses on monocytes as the cell type with most robust baseline expression of IFNα/βR expression (allowing clearer detection of inhibition) and greatest numbers of cells available for other investigations. Peripheral blood CD4+ T-cell count was significantly associated with monocyte IFNα/βR sMFI in HIV-1–infected subjects ($r = 0.545$, $P < .001$; Figure 2A), and plasma HIV-1 RNA level was inversely correlated with monocyte IFNα/βR sMFI ($r = -0.577$, $P < .001$; Figure 2B). Because expression of CD38 on memory CD8+ T cells is a marker of chronic immune activation and strongly associated with HIV-1 disease progression,[23-25] we also analyzed the relationship of IFNα/βR expression on monocytes with expression of CD38 on memory (CD45RO+) CD8+ T cells (the memory subset is targeted as CD38 is expressed on naive CD8+ T cells in the absence of activation).[27,28] We found a significant inverse relationship between sMFI for CD38 expression on memory CD8+ T cells and sMFI for IFNα/βR on monocytes ($r = -0.620$, $P < .001$; Figure 2C). In uninfected subjects, there was not a significant correlation between CD38 expression on memory CD8+ T cells and monocyte IFNα/βR expression ($r = 0.437$, $P = .119$). Thus, monocyte IFNα/βR expression correlated with markers of HIV-1 disease stage and level of immune activation in HIV-1 infection.

Because CD4+ T-cell count, HIV-1 RNA, and memory CD8+ T-cell CD38 expression were each associated with IFNα/βR expression, we examined whether these variables were independent predictors of IFNα/βR expression level (Table 1). Multivariate regression analysis showed that CD38 expression on memory CD8+ T cells independently predicted IFNα/βR expression on monocytes among HIV-1–infected subjects after controlling for CD4+ T-cell count and HIV-1 RNA levels in plasma ($P = .013$), whereas CD4+ T-cell count and HIV-1 RNA levels in plasma were not independent predictors of IFNα/βR expression after accounting for CD38 expression on memory CD8+ T cells. The independent relationship between CD38 expression by memory CD8+ T cells and IFNα/βR suggests that diminished IFNα/βR expression on monocytes may be an especially important marker of chronic

Table 1. Monocyte IFNα/βR expression is independently associated with CD38 expression by memory CD8+ T cells

Control variable	Correlated variable		
	CD8+ T-cell CD38 expression	HIV-1 RNA load	CD4 T-cell count
CD4	−0.582	−0.260	
P	.010	.065	
HIV-1 RNA	−0.726		0.494
P	< .001		< .001
CD4 and HIV-1 RNA	−0.556		
P	.013		
CD38		−0.22	0.383
P		.351	.095
HIV-1 RNA and CD38			0.322
P			.179
CD4 and CD38		−0.001	
P		.997	

Multivariate analysis was used to determine associations between monocyte IFNα/βR expression and clinical markers of disease progression. Analysis was restricted to the subset of HIV-infected subjects for whom CD4+ T-cell count, plasma HIV RNA level, and CD38 expression by memory CD8+ T cells were all measured (n = 22). Data are shown as the partial correlation coefficients of IFNα/βR expression with each of the correlated variables shown in columns after controlling for each of the variables shown in rows. The association of IFNα/βR expression with CD38 expression by memory CD8+ T cells changes minimally after controlling for CD4+ T-cell count, HIV-1 RNA level, or both.
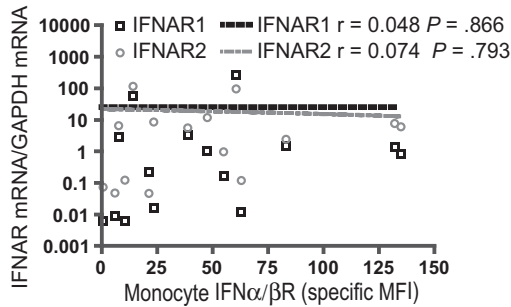
Figure 3. Loss of IFNα/βR protein expression at the cell surface is not explained by diminished IFNα/βR mRNA. RNA was extracted from purified monocytes from uninfected and HIV-1–infected persons ex vivo, and mRNA for IFNAR1 and IFNAR2 (both chains of the IFNα/βR) was quantified by qRT-PCR and normalized to GAPDH. Cell-surface IFNα/βR expression, as determined by flow cytometry, did not correlate with mRNA for IFNAR1 (r = 0.048, P = .866) or IFNAR2 (r = 0.074, P = .793).

immune activation and may be associated with subsequent disease progression, although this hypothesis remains to be tested.

Monocyte IFNα/βR expression is regulated at the translational or posttranslational level

Processes that may lead to loss of IFNα/βR expression include a variety of mechanisms from transcriptional regulation to posttranslational regulation, such as ligand-induced receptor internalization and degradation. We used qRT-PCR to quantify mRNA for the 2 subunits of the IFNα/βR, IFNAR1 and IFNAR2, in purified monocytes (n = 17). Univariate linear regression analysis showed that IFNα/βR protein expression did not correlate with IFNAR1 (r = 0.048, P = .866) or IFNAR2 (r = 0.074, P = .793) mRNA (Figure 3). For example, some persons with substantial loss of cell-surface IFNα/βR protein had IFNAR1 and IFNAR2 mRNA levels similar to those of HIV-1–infected persons with higher IFNα/βR protein expression. These data suggest that IFNα/βR expression is diminished by posttranslational mechanisms in HIV-1 infection.

We considered the hypothesis that in vivo exposure to IFNα/β may contribute to loss of IFNα/βR expression, consistent with prior observations of ligand-induced posttranslational degradation of IFNα/βR. Plasma IFNα levels were assessed by ELISA for 22 HIV-1–infected subjects and 6 uninfected persons. Plasma IFNα levels in uninfected persons (median, 10.6 pg/mL; IQR 1.2-19.5 pg/mL) and HIV-1–infected subjects (median, 5.4 pg/mL; IQR, 2.5-10.8 pg/mL) were close to or below the threshold of detectability and were not significantly different (P = .502; data not shown). Although significant detection of plasma IFNα was not achieved in these assays, ligand-induced down-regulation of IFNα/βR may still result from either exposure of cells to IFNα at different times or anatomical sites, or the effects of other type I IFN species (eg, IFNβ or IFNω).

IFNα/β-stimulated gene induction is significantly impaired by multiple mechanisms in monocytes of HIV-1–infected subjects

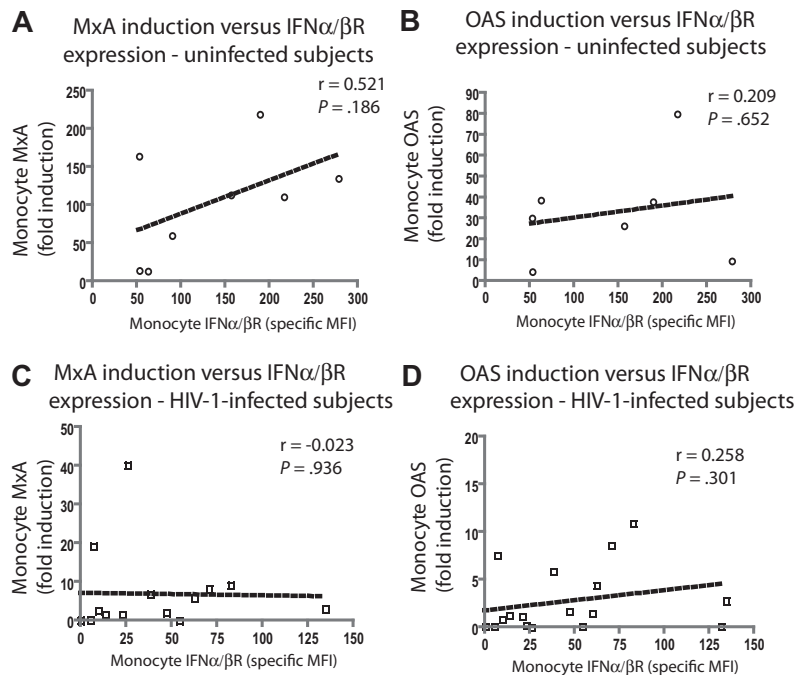To investigate the functional relevance of diminished monocyte IFNα/βR, we tested the ability of IFNα to induce expression of 2 classical ISG produces MxA and OAS, in monocytes from HIV-1–infected subjects and uninfected persons. ISG induction was assessed by qRT-PCR of mRNA isolated from purified monocytes either directly ex vivo or after incubation for 18 hours with or without 1000 U/mL IFNα2a. Fold induction was calculated
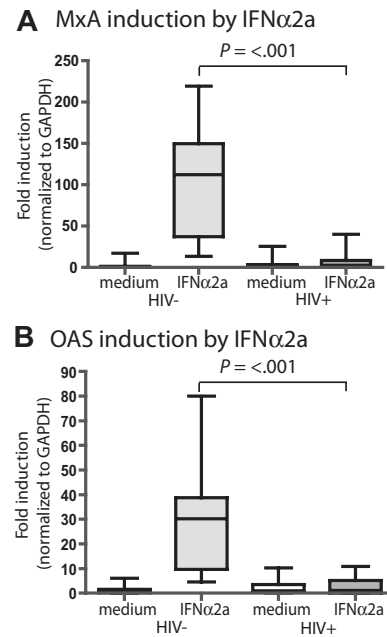
Figure 4. Impaired induction of MxA and OAS in monocytes from HIV-1–infected persons. RNA was extracted from purified monocytes from uninfected and HIV-1–infected persons ex vivo or after culture for 18 hours with 1000 U/mL IFNα2a or without IFNα2a ("medium"), and mRNA for MxA or OAS was quantified by qRT-PCR and normalized to GAPDH. Results are expressed as fold induction (expression after 18-hour incubation versus ex vivo). Fold induction was significantly reduced for MxA mRNA (P < .001; A) and OAS mRNA (P < .001; B) in monocytes of HIV-1–infected persons in comparison to uninfected subjects. Boxes show median, 25th and 75th percentile values and whiskers show ranges.

as mRNA expression after stimulation divided by mean baseline mRNA expression in unstimulated monocytes (ex vivo). IFNα2a induced a substantial increase in expression of MxA in monocytes of uninfected persons (n = 9; Figure 4A), but this induction was significantly impaired in monocytes from HIV-1–infected subjects (n = 18; Figure 4A). The median fold induction of MxA mRNA in monocytes was 112.3 (IQR, 37.3-149.8) for uninfected persons versus 2.6 (IQR, 1.5-8.2) for HIV-1–infected subjects (P < .001). A similar pattern was observed for induction of OAS mRNA (Figure 4B). The median fold induction of OAS in monocytes was 30.2 (IQR, 9.6-38.8) in uninfected persons versus 1.1 (IQR, 0.1-4.4) in HIV-1–infected subjects (P < .001). Thus, the ability of IFNα2a to induce MxA and OAS was impaired significantly in monocytes of HIV-1–infected subjects.

Multiple regression analysis was used to assess the relationship between IFNα/βR expression and the induction of ISGs by IFNα. Although HIV-1 status was strongly and independently associated with the magnitude of IFNα-induction of MxA (P = .008) and OAS (P = .002), linear regression analysis of HIV-1–infected subjects showed no significant relationship between expression of IFNα/βR and magnitude of induction of MxA (Figure 5C; n = 15; r = −0.023, P = .936) or OAS (Figure 5D; n = 18; r = 0.258, P = .301). These results do not exclude IFNα/βR expression as a contributor to loss of IFNα/β responsiveness in HIV-1 infection, but they suggest that inhibition of post-IFNα/βR signaling mechanisms may contribute significantly to limiting induction of MxA and OAS. In uninfected persons there was also a lack of correlation between IFNα/βR expression and induction of MxA (Figure 5A; n = 8; r = 0.521, P = .186) or OAS (Fig 5B; n = 7; r = 0.209, P = .652), probably because of sufficient expression of receptors beyond a level that would limit responses to IFNα/β. We conclude that loss of IFNα/βR is associated with disease progression, but

A MxA induction versus IFNα/βR expression - uninfected subjects

B OAS induction versus IFNα/βR expression - uninfected subjects

C MxA induction versus IFNα/βR expression - HIV-1-infected subjects

D OAS induction versus IFNα/βR expression - HIV-1-infected subjects

Figure 5. Deficits in ISG induction are not significantly correlated with deficits in IFNα/βR expression. Univariate linear regression analysis was used to assess relationships of ISG gene induction (Figure 4) with IFNα/βR expression. For uninfected subjects, induction of MxA (A) and OAS (B) was not significantly related to monocyte IFNα/βR expression ($P = .186$ and $P = .652$, respectively). Similarly, for HIV-1-infected subjects, induction of MxA (C) and OAS (D) was not significantly related to IFNα/βR expression ($P = .936$ and $P = .301$, respectively).

inhibition of postreceptor signaling mechanisms may also contribute to loss of specific IFN-induced effects.

IFN-induced STAT1 phosphorylation is impaired in monocytes of HIV-1-infected persons independent of IFNα/βR expression

Because factors other than decreased IFNα/βR expression were implicated in the diminished induction of MxA and OAS by IFNα in HIV-1 infection (Figure 5), we assessed other steps in IFNα/β signaling, including STAT1 tyrosine phosphorylation. PBMCs from HIV-1-infected and uninfected persons were stimulated with IFNα2a at 0, 1000, 3000, and 10 000 U/mL for 15 minutes, fixed, and permeabilized for intracellular staining and flow cytometric detection of phosphorylated STAT1 (pSTAT1)[29] in gated monocytes. IFNα2a induced phosphorylation of STAT1 in monocytes of uninfected persons (Figure 6A), but monocytes from HIV-1-infected subjects were substantially deficient in STAT1 phosphorylation at each concentration of IFNα2a (Figure 6B). The delta (Δ) pSTAT1 sMFI was calculated as the difference in sMFI between cells incubated in the presence of IFNα2a and cells incubated in medium alone. Substantial dose-dependent pSTAT1 responses were evident in monocytes from uninfected persons with median Δ sMFI of 76 (IQR, 44-100) at 1000 U/mL IFNα2a, 124 (IQR, 97-132) at 3000 U/mL IFNα2a, and 136 (IQR, 128-144) at 10 000 U/mL IFNα2a (Figure 6C). In contrast, STAT1 phosphorylation was impaired in monocytes from HIV-1-infected subjects with median Δ sMFI of 8 (IQR, 2-29) at 1000 U/mL, 20 (IQR, 7-65) at 3000 U/mL, and 57 (IQR, 20-126) at 10 000 U/mL IFNα2a (Figure 6C). The difference in monocyte Δ pSTAT1 sMFI between uninfected and HIV-1-infected subjects was statistically significant at concentrations of 1000 U/mL ($P = .012$) and at 3000 U/mL ($P = .005$), but not at 10 000 U/mL ($P = .075$) of IFNα2a. The greatest difference between HIV-1-infected and uninfected persons was at 3000 U/mL IFNα2a, a concentration on the rising phase of the dose-response curve. We conclude that IFNα-induced phosphorylation of STAT1 was significantly impaired in monocytes from HIV-1-infected subjects.

We examined the relationship between induction of pSTAT1 and cell-surface IFNα/βR expression. The Δ pSTAT1 sMFI at all

3 concentrations of IFNα2a was compared with cell-surface IFNα/βR expression by linear regression. For uninfected persons, monocytes showed various levels of IFNα/βR expression and IFNα2a-induced pSTAT1, but the levels of IFNα/βR expression and STAT1 phosphorylation were not significantly correlated at 1000 U/mL ($r = 0.114$, $P = .789$; data not shown), 3000 U/mL ($r = -0.318$, $P = .443$; Figure 7A), or 10 000 U/mL ($r = 0.481$, $P = .275$) IFNα2a (data not shown). Analysis of monocytes from HIV-1-infected subjects similarly showed that monocyte expression of IFNα/βR was not correlated with phosphorylation of STAT1 at 1000 U/mL ($r = 0.424$, $P = .131$; data not shown), 3000 U/mL ($r = 0.330$, $P = .249$; Figure 7B), or 10 000 U/mL ($r = 0.153$, $P = .618$) IFNα2a (data not shown). These data indicate that the magnitude of STAT1 phosphorylation was influenced by factors other than the level of IFNα/βR expression. For example, ablation of IFNα2a-induced STAT1 phosphorylation was observed in a subset of HIV-1-infected subjects despite intermediate or high level IFNα/βR expression. Therefore, failure of IFNα2a to induce tyrosine phosphorylation of STAT1 may stem at least in part from inhibitory signaling mechanisms other than loss of cell-surface IFNα/βR. These data imply that impairment of monocyte responses to IFNα/β in HIV-1 infection results from defects at multiple signaling levels and is not completely explained by down-regulation of the IFNα/βR.

Discussion

In this study we have shown significant loss of IFNα/β responsiveness in monocytes from HIV-1-infected persons that is manifested at multiple levels of the IFNα/β signaling pathway, including loss of IFNα/βR ($P < .001$), IFNα-induced STAT1 phosphorylation ($P = .005$), and IFNα induction of MxA and OAS mRNA (both $P < .001$). Early in the course of the HIV pandemic, loss of IFNα/βR expression was noted on unfractionated PBMCs of HIV-1-infected persons, with symptomatic disease defined as AIDS or AIDS-related complex,[30] but there is little recent information to assess cell type-specific mechanisms, to provide functional
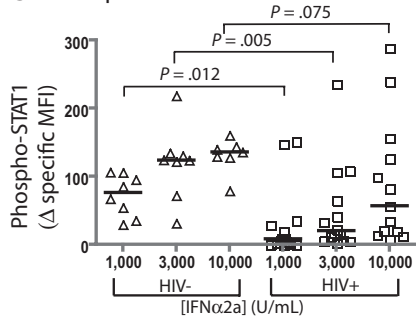
A Phospho-STAT1 induction, uninfected individuals
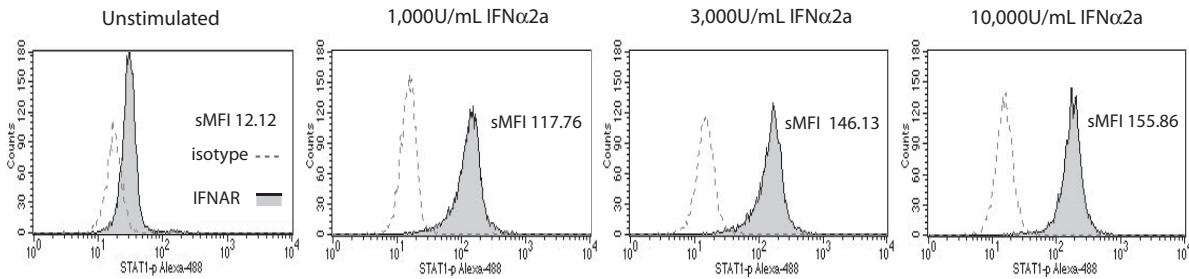
B Phospho-STAT1 induction, HIV-1–infected individuals
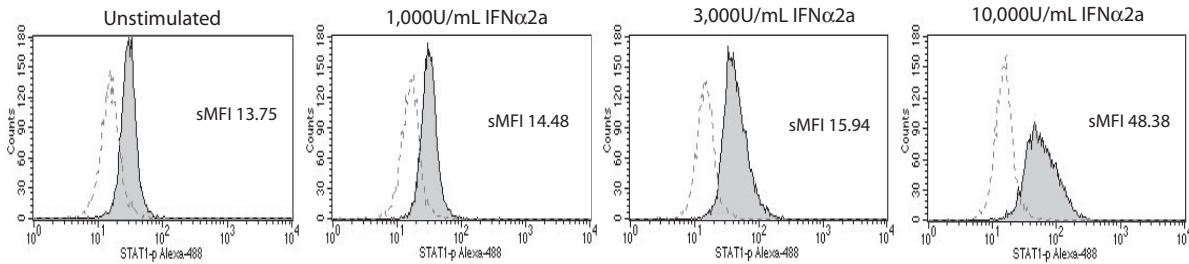
C Phospho-STAT1 induction

Figure 6. Induction of phosphorylated STAT1 by IFNα2a is inhibited in monocytes from HIV-1–infected persons. Monocytes were incubated for 15 minutes with IFNα2a at 0, 1000, 3000, or 10 000 U/mL. Individual histograms are shown for uninfected (A) and HIV-1–infected (B) subjects. Dose response induction of phosphorylated STAT1 is evident in uninfected persons but is inhibited in HIV-1–infected persons (C). The difference in phosphorylated STAT1 response between uninfected and HIV-1–infected subjects is significant at 1000 U IFNα2a (P = .012) and 3000 U IFNα2a (P = .005) but not at 10 000 U IFNα2a (P = .075). Results are expressed as Δ sMFI (sMFI of stimulated monocytes − sMFI of unstimulated monocytes). Horizontal bars in panel C represent median values.

data on IFNα/β responsiveness, or to determine associations with markers of disease progression. Our studies indicate that there is a profound impairment in IFNα/β signaling in monocytes obtained from HIV-1 infection even during the asymptomatic phase of infection. Although defects at multiple stages of IFNα/β signaling may contribute, loss of IFNα/βR provides a potentially powerful and practical indicator of pathologic immune activation in HIV-1 infection that correlates well with markers of disease progression.

Deficits in IFNα/βR expression, STAT1 phosphorylation, and ISG induction were observed similarly in male and female persons

with HIV-1 infection. There were no significant sex differences for sMFI or percentage of positive expression of IFNα/βR on monocytes, mDCs, or pDCs ex vivo. In addition, there were no significant sex differences for induction of pSTAT1 or mRNA for MxA or OAS in monocytes exposed to IFNα2a.

Results from this study indicate a widespread loss of IFNα/β responsiveness in monocytes. We do not find evidence for dichotomous responses of different cell subpopulations to IFNα/β that would suggest the presence of both responsive and nonresponsive monocyte subsets. In all flow cytometry–based assays, IFNα/βR
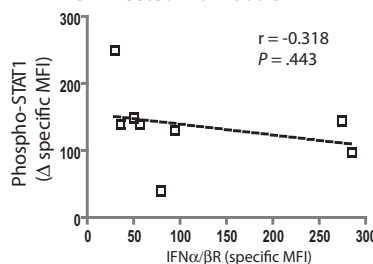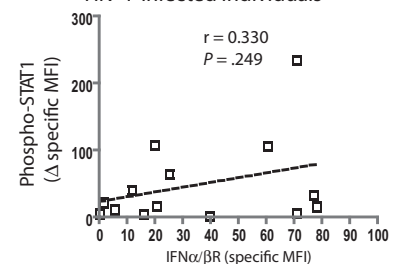
Figure 7. Induction of phosphorylated STAT1 by IFNα2a is not related to IFNα/βR expression in monocytes. Univariate linear regression analysis was used to assess the relationship between IFNα/βR expression and capacity to induce STAT1 phosphorylation. Significant correlation was not observed between induction of phosphorylated STAT1 and expression of IFNα/βR for uninfected persons (r = −0.318, P = .443; A) or HIV-1–infected persons (r = 0.330, P = .249; B).

A Phospho-STAT1 induction (3,000U/mL IFNα2a) Uninfected individuals

r = −0.318
P = .443

B Phospho-STAT1 induction (3,000U/mL IFNα2a) HIV-1-infected individuals

r = 0.330
P = .249

expression and induction of phosphorylated STAT1 were consistently represented by single populations, as can be seen in the flow histograms in Figures 1 and 6.

Significant loss of IFNα/βR expression (sMFI) in HIV-1–infected persons was detected in monocytes, mDCs, and pDCs. We found no evidence for reduced IFNα/βR expression on T cells. Monocytes provide a particularly robust system for analysis of IFNα/βR in HIV infection, because they express higher baseline levels of IFNα/βR (allowing clearer detection of inhibition), and their abundance allows a range of analyses. Thus, IFNα/βR expression on monocytes may be both important and pragmatic as a marker that correlates with disease progression.

Mechanisms other than receptor loss may contribute to impairment of IFNα/β responsiveness in HIV-1 infection. IFNα-induced STAT1 phosphorylation was impaired significantly in monocytes of HIV-1–infected subjects (Figure 6), and these monocytes failed to increase expression of the IFN-stimulated genes MxA and OAS after exposure to exogenous IFNα (Figure 4). Deficits in IFNα2a-induced STAT1 phosphorylation and induction of MxA and OAS were not significantly related to levels of IFNα/βR, indicating contributions of inhibitory mechanisms in addition to the loss of cell-surface IFNα/βR. Alhetheel et al[31] recently reported elevation of total STAT1 and IFNγ-induced STAT1 phosphorylation, but not IFNα-induced STAT1 phosphorylation, in monocytes from HIV-1–infected subjects, suggesting that STAT1 phosphorylation defects may be specific to the IFNα/β signaling pathway. Mechanisms that could reduce STAT1 signaling include induction or activation of suppressor of cytokine synthesis (SOCS) molecules[32] or protein inhibitor of activated STATs (PIAS),[33] which inhibit induction of phosphorylated STAT1 or its activity, respectively. Thus, impairment of monocyte responses to IFNα/β in HIV-1 infection may be multifactorial, resulting from cumulative deficits at more than one signaling level.

Because IFNα/β is known to decrease expression of IFNα/βR by ligand-induced receptor degradation, one hypothesis to explain the loss of IFNα/β responsiveness in HIV-1 infection is that chronic exposure to IFNα/β results in desensitization. A chronic period of exposure at potentially suboptimal levels of IFNα/β may contribute to desensitization of the response. We did not detect plasma levels of IFNα sufficient to determine any relationship with IFNα/βR expression by monocytes, although these assays may lack sufficient sensitivity and may not detect IFNα potentially expressed at different times or anatomical sites, or the effects of IFNβ or IFNω. Other reports provide evidence that chronic exposure to IFNα/β occurs in HIV-1 infection, resulting in increased expression of ISGs[34] and influencing turnover of uninfected CD4+ T cells.[21] Thus, a role for ligand-induced receptor degradation is still possible. The finding that IFNα/βR mRNA did not correlate with IFNα/βR expression suggests a role for translational or posttranslational regulation, consistent with the hypothesis that exposure to IFNα/β induces degradation of monocyte IFNα/βR in HIV-1 infection, but this remains to be tested.

HIV-1 infection is associated with increased levels of microbial ligands for innate immune receptors that may regulate IFNα/β production and responsiveness. Systemic immune activation may result from exposure to HIV-1 RNA sequences that bind TLR7 or TLR8, or exposure to bacterial products such as the TLR4 agonist, LPS, that are translocated across damaged gut epithelium.[22,35-37] Chronic TLR signaling in HIV-1 infection may result in chronic expression of IFNα/β, which may induce some genes and proteins (eg, CD38 expression on CD8+ T cells[19]) but may also result in

decreased expression of IFNα/βR and desensitization to IFNα/β. In addition, TLR signaling may induce expression or activity of SOCS, PIAS, or other negative regulators of IFNα/β signaling, providing another mechanism for decreased IFNα/β responsiveness. Thus, changes in IFNα/βR expression and IFNα/β responsiveness may correlate with TLR-dependent systemic immune activation in general and monocyte activation specifically. We propose that IFNα/βR down-regulation may be a powerful indicator of pathologic immune activation and disease progression, although this hypothesis requires further study.

Our studies showed significant correlations between IFNα/βR expression level and markers of disease progression in HIV-1 infection. Monocyte IFNα/βR correlated directly with CD4+ T-cell count and inversely with plasma HIV-1 RNA levels and expression of CD38 on memory CD8+ T cells (a marker of pathologic immune activation that is prognostic for disease progression[24,25]). Although CD4+ T-cell count, HIV-1 RNA, and CD8+ T-cell immune activation (as manifested by CD38 expression) correlated individually with IFNα/βR expression, multivariate analysis indicated that only CD38 expression on CD8+ T cells was independently associated with IFNα/βR expression (Table 1), suggesting that factors in HIV-1 infection that drive pathologic immune activation may be more closely related to monocyte IFNα/βR expression than to plasma HIV-1 RNA level or CD4+ T-cell count. We have previously shown that IFNα treatment up-regulates CD38 expression, especially on CD8 T cells of HIV-1–infected persons,[19] which suggests that IFNα/β may provide a common pathway to both monocyte IFNα/βR loss and expression of CD38 on memory CD8+ T cells. Therefore, we propose that IFNα/βR expression on monocytes may be a novel, highly predictive marker for disease progression (either alone or in combination with CD38 expression on CD8+ T cells), a hypothesis that must be addressed in future longitudinal studies. Impairment of IFNα/β signaling may contribute significantly to immunopathogenesis of HIV-1 infection in several ways, including potential attenuation of antiviral defenses and antigen-presenting cell maturation and activation.

Authorship

Contribution: G.A.D.H. contributed to experimental design and performed most of the experiments, data analysis, and manuscript preparation; S.F.S. assisted in experimental design, assay development, data analysis, and manuscript preparation; B.R. assisted in study design, statistical analysis, and manuscript preparation; W.J.

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References

1. Boasso A, Shearer GM. Chronic innate immune activation as a cause of HIV-1 immunopathogenesis. Clin Immunol. 2008;126:235-242.

2. Donaghy H, Pozniak A, Gazzard B, et al. Loss of blood CD11c(+) myeloid and CD11c(-) plasmacytoid dendritic cells in patients with HIV-1 infection correlates with HIV-1 RNA virus load. Blood. 2001;98:2574-2576.

3. Shortman K, Liu YJ. Mouse and human dendritic cell subtypes. Nat Rev Immunol. 2002;2:151-161.

4. Kadowaki N, Liu YJ. Natural type I interferon-producing cells as a link between innate and adaptive immunity. Hum Immunol. 2002;63:1126-1132.

5. Ehlers M, Ravetch JV. Opposing effects of Toll-like receptor stimulation induce autoimmunity or tolerance. Trends Immunol. 2007;28:74-79.

6. Jiang W, Lederman MM, Salkowitz JR, Rodriguez B, Harding CV, Sieg SF. Impaired monocyte maturation in response to CpG oligodeoxynucleotide is related to viral RNA levels in human immunodeficiency virus disease and is at least partially mediated by deficiencies in alpha/beta interferon responsiveness and production. J Virol. 2005;79:4109-4119.

7. Mogensen KE, Lewerenz M, Reboul J, Lutfalla G, Uze G. The type I interferon receptor: structure, function, and evolution of a family business. J Interferon Cytokine Res. 1999;19:1069-1098.

8. O'Shea JJ, Notarangelo LD, Johnston JA, Candotti F. Advances in the understanding of cytokine signal transduction: the role of Jaks and STATs in immunoregulation and the pathogenesis of immunodeficiency. J Clin Immunol. 1997;17: 431-447.

9. Colonna M, Trinchieri G, Liu YJ. Plasmacytoid dendritic cells in immunity. Nat Immunol. 2004;5: 1219-1226.

10. Siegal FP, Kadowaki N, Shodell M, et al. The nature of the principal type 1 interferon-producing cells in human blood. Science. 1999;284:1835-1837.

11. 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.

12. Santini SM, Di Pucchio T, Lapenta C, Parlato S, Logozzi M, Belardelli F. The natural alliance between type I interferon and dendritic cells and its role in linking innate and adaptive immunity. J Interferon Cytokine Res. 2002;22:1071-1080.

13. Honda K, Sakaguchi S, Nakajima C, et al. Selective contribution of IFN-alpha/beta signaling to the maturation of dendritic cells induced by double-stranded RNA or viral infection. Proc Natl Acad Sci U S A. 2003;100:10872-10877.

14. Lane HC, Davey V, Kovacs JA, 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.

15. Kovacs JA, Bechtel C, Davey RT Jr, et al. Combination therapy with didanosine and interferon-alpha in human immunodeficiency virus-infected patients: results of a phase I/II trial. J Infect Dis. 1996;173:840-848.

16. 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.

17. Khatissian E, Tovey MG, Cumont MC, et al. The relationship between the interferon alpha response and viral burden in primary SIV infection. AIDS Res Hum Retroviruses. 1996;12:1273-1278.

18. 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.

19. Rodriguez B, Lederman MM, Jiang W, et al. Interferon-alpha differentially rescues CD4 and CD8 T cells from apoptosis in HIV infection. AIDS. 2006; 20:1379-1389.

20. Herbeuval JP, Nilsson J, Boasso A, et al. Differential expression of IFN-alpha and TRAIL/DR5 in lymphoid tissue of progressor versus nonprogressor HIV-1-infected patients. Proc Natl Acad Sci U S A. 2006;103:7000-7005.

21. Sedaghat AR, German J, Teslovich TM, 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.

22. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med. 2006;12:1365-1371.

23. Liu Z, Cumberland WG, Hultin LE, Kaplan AH, Detels R, Giorgi JV. CD8+ T-lymphocyte activation in HIV-1 disease reflects an aspect of pathogenesis distinct from viral burden and immunodeficiency. J Acquir Immune Defic Syndr Hum Retrovirol. 1998;18:332-340.

24. Liu Z, Cumberland WG, Hultin LE, Prince HE, Detels R, Giorgi JV. Elevated CD38 antigen expression on CD8+ T cells is a stronger marker for the risk of chronic HIV disease progression to AIDS and death in the Multicenter AIDS Cohort Study than CD4+ cell count, soluble immune activation markers, or combinations of HLA-DR and CD38 expression. J Acquir Immune Defic Syndr Hum Retrovirol. 1997;16:83-92.

25. Giorgi JV, Hultin LE, McKeating JA, et al. Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. J Infect Dis. 1999;179:859-870.

26. Vitale G, de Herder WW, van Koetsveld PM, et al. IFN-beta is a highly potent inhibitor of gastroenteropancreatic neuroendocrine tumor cell growth in vitro. Cancer Res. 2006;66:554-562.

27. Prince HE, York J, Jensen ER. Phenotypic comparison of the three populations of human lymphocytes defined by CD45RO and CD45RA expression. Cell Immunol. 1992;145:254-262.

28. Bofill M, Mocroft A, Lipman M, et al. Increased numbers of primed activated CD8+CD38+CD45RO+ T cells predict the decline of CD4+ T cells in HIV-1-infected patients. AIDS. 1996;10:827-834.

29. Perez OD, Nolan GP. Phospho-proteomic immune analysis by flow cytometry: from mechanism to translational medicine at the single-cell level. Immunol Rev. 2006;210:208-228.

30. Lau AS, Read SE, Williams BR. Downregulation of interferon alpha but not gamma receptor expression in vivo in the acquired immunodeficiency syndrome. J Clin Invest. 1988;82:1415-1421.

31. Alhetheel A, Yakubtsov Y, Abdkader K, et al. Amplification of the signal transducer and activator of transcription I signaling pathway and its association with apoptosis in monocytes from HIV-infected patients. AIDS. 2008;22:1137-1144.

32. Alexander WS. Suppressors of cytokine signalling (SOCS) in the immune system. Nat Rev Immunol. 2002;2:410-416.

33. O'Shea JJ, Watford W. A peek at PIAS. Nat Immunol. 2004;5:875-876.

34. Tilton JC, Johnson AJ, Luskin MR, 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.

35. Guadalupe M, Reay E, Sankaran S, et al. Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. J Virol. 2003;77:11708-11717.

36. Li Q, Duan L, Estes JD, et al. Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. Nature. 2005;434: 1148-1152.

37. Mattapallil JJ, Douek DC, Hill B, Nishimura Y, Martin M, Roederer M. Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. Nature. 2005;434:1093-1097.