Monocyte and Myeloid Dendritic Cell Activation Occurs Throughout HIV Type 2 Infection, an Attenuated Form of HIV Disease

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Monocytes and myeloid dendritic cells (mDCs) are important orchestrators of innate and human immunodeficiency virus (HIV)–specific immune responses and of the generalized inflammation that characterizes AIDS progression. To our knowledge, we are the first to investigate monocyte and mDC imbalances in HIV type 2 (HIV-2)–positive patients, who typically feature reduced viremia and slow disease progression despite the recognized ability of HIV-2 to establish viral reservoirs and overcome host restriction factors in myeloid cells. We found a heightened state of monocyte and mDC activation throughout HIV-2 infection (characterized by CD14brightCD16+ expansion, as well as increased levels of soluble CD14, HLA-DR, and CD86), together with progressive mDC depletion. Importantly, HIV-2–positive patients also featured overexpression of the inhibitory molecule PD-L1 on monocytes and mDCs, which may act by limiting the production of proinflammatory molecules. These data, from patients with a naturally occurring form of attenuated HIV disease, challenge current paradigms regarding the role of monocytes in HIV/AIDS and open new perspectives regarding potential strategies to modulate inflammatory states.

Keywords. HIV-2; AIDS; Monocytes; Myeloid Dendritic Cells; PD-L1; Immune Activation.

Human immunodeficiency virus (HIV) infection leads to a state of heightened immune activation, which is considered a main determinant of the associated progressive immunodeficiency [1]. Monocytes/macrophages and myeloid dendritic cells (mDCs) are key producers of proinflammatory cytokines and chemokines, thereby directing activation and differentiation of adaptive immune responses and recruiting T cells to sites of viral replication [2]. HIV type 1 (HIV-1) infection has been consistently associated with expansion of CD16+ monocytes that have variable levels of CD14 expression [3–6] and are highly susceptible to infection and preferentially harbor HIV for the long term [7, 8]. Recent in vivo studies have shown that, at inflammatory sites, monocytes can differentiate into mDCs [9], professional antigen-presenting cells known to be depleted and functionally impaired in HIV-1 infection [10–12]. The mechanisms underlying monocyte and mDC disturbances during HIV-1 infection remain largely unknown.

HIV type 2 (HIV-2) infection is a naturally occurring form of attenuated HIV disease, characterized by slow CD4+ T-cell decline, undetectable-to-low levels of circulating virus, and limited impact on the mortality...
of infected adults [13]. This is despite similar levels of proviral DNA in HIV-2–positive and HIV-1–positive individuals, indicating a comparable number of infected cells and ability of both viruses to disseminate and establish viral reservoirs [14, 15].

The reduced HIV-2 viremia is thought to account for the low rates of horizontal and vertical transmission and the confinement of HIV-2 infection to West Africa and related countries, such as Portugal [16].

The reasons for the better prognosis of HIV-2 disease remain elusive [17]. We have shown that chronic immune activation is a major driving force for CD4+ T-cell depletion during HIV-2 infection [18], as has been reported for HIV-1 [1]. Nevertheless, it is important to emphasize that the rate of increase in immune activation and the linked rate of decline in CD4+ T-cell count are much slower in HIV-2 disease, compared with HIV-1 disease [19].

Data regarding mDCs and monocytes in the context of HIV-2 infection are scarce. Duvall et al reported that mDCs were less susceptible to HIV-2 than HIV-1 infection in vitro [20]. Notwithstanding this finding, recent evidence supports a unique ability of HIV-2 to overcome host restriction factors limiting HIV-1 replication in myeloid cells [21]. Moreover, although HIV-1 envelope proteins per se are able to induce mDC disturbances [22], we showed that HIV-2 envelope protein did not alter the capacity of monocytes to differentiate into DCs and subsequent DC maturation [23]. Nevertheless, our previous data showed that HIV-2 envelope protein induced tumor necrosis factor α (TNF-α) production by monocytes [24, 25] and suppressed T-cell proliferation through a monocyte-mediated mechanism [25].

Here, we provide the first comprehensive study of monocytes and mDCs in untreated HIV-2–positive individuals. Our data provide evidence of a heightened state of monocyte activation throughout HIV-2 infection, which is accompanied by upregulation of the inhibitory molecules PD-L1/PD-1, possibly contributing to the relatively “benign” outcome of HIV-2 infection.

**METHODS**

**Studied Cohorts**

Clinical and epidemiological data are shown in Table 1. HIV-2 and HIV-1 cohorts were antiretroviral therapy naive, had no ongoing clinical opportunistic infections or tumors, and featured comparable degrees of CD4+ T-cell depletion. Accordingly, both cohorts exhibited similar activation marker upregulation among CD4+ and CD8+ T cells, as previously reported [18]. Notably, 21 of 29 HIV-2–positive patients had undetectable viremia, with the highest documented level (26 263 RNA copies/mL) significantly lower than levels among HIV-1–positive individuals. Nevertheless, comparable levels of proviral DNA and subsequent DC maturation [23].

**Table 1. Clinical and Epidemiological Characteristics of Seronegative, Human Immunodeficiency Virus Type 1 (HIV-1)–Positive, and HIV Type 2 (HIV-2)–Positive Individuals**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Seronegative (n=17)</th>
<th>HIV-1 (n=22)</th>
<th>HIV-2 (n=29)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>6</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>11</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>Age, y</td>
<td>44 (27–57)</td>
<td>38 (23–61)</td>
<td>52 (19–78)</td>
<td>&lt;.05a</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>White</td>
<td>15</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>2</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>CD4+ T-cell %</td>
<td>43.2 (34.4–61.1)</td>
<td>19.3 (1.2–47.2)</td>
<td>24.7 (7.1–54.1)</td>
<td>&lt;.001b; &lt;.001c</td>
</tr>
<tr>
<td>CD4+ T-cell count, cells/µL</td>
<td>821 (618–1312)</td>
<td>358 (18–1848)</td>
<td>538 (52–1511)</td>
<td>&lt;.01b; &lt;.05c</td>
</tr>
<tr>
<td>HLA-DR+ % (CD4)</td>
<td>3.9 (1.9–7.6)d</td>
<td>15.2 (1.7–54.5)</td>
<td>9.4 (1.9–36.3)</td>
<td>&lt;.001b; &lt;.001c</td>
</tr>
<tr>
<td>HLA-DR+CD38+ % (CD8)</td>
<td>2.7 (1.3–22.7)d</td>
<td>25.6 (1.4–62.2)</td>
<td>14.3 (0.6–69.5)</td>
<td>&lt;.001b; &lt;.001c</td>
</tr>
<tr>
<td>Viremia, RNA copies/mL</td>
<td>NA</td>
<td>14 094 (40–4.5 × 10⁶)e</td>
<td>200 (200–26 263)e</td>
<td>&lt;.01a</td>
</tr>
<tr>
<td>Proviral DNA level, copies/10⁶ PBMCs</td>
<td>NA</td>
<td>68 (5–975) ¹</td>
<td>5 (5–1033)g</td>
<td></td>
</tr>
</tbody>
</table>

Data are no. of individuals or median value (range). HIV-positive patients were followed at the Clínica Universitária de Doenças Infecciosas, Hospital de Santa Maria, Faculdade de Medicina da Universidade de Lisboa.

Abbreviation: NA, not applicable.

a HIV-1 vs HIV-2.
b Seronegative vs HIV-1.
c Seronegative vs HIV-2.
d Data are for 16 individuals.
e Four HIV-1–positive patients and 21 HIV-2–positive patients had undetectable viremia. In these cases, the cutoff value of the test (40 and 200 RNA copies/mL for HIV-1 and HIV-2, respectively) was used to calculate the median.

f Data are for 21 individuals.
g Data are for 25 individuals.
DNA were found in peripheral blood mononuclear cells (PBMCs), in agreement with previous reports [14, 15]. Of note, 4 HIV-1–positive individuals had undetectable viremia (<40 RNA copies/mL) and met the formal definition of “elite controllers,” a group that usually composes <1% of the HIV-1–positive population [26]. All individuals gave written informed consent for blood sample collection and processing. The study was approved by the Ethical Board of the Faculty of Medicine, University of Lisbon.

Cell Isolation and Cell Culture
PBMCs were isolated from heparinized blood immediately after venipuncture, using the Ficoll-Hypaque (Sigma) technique. Monocytes were purified using magnetic beads (purity, >98%; StemCell). In vitro responses to lipopolysaccharide (LPS) were evaluated upon culture of 2 × 10^6 PBMCs/mL in 24-well plates with complete medium [27], in the absence or presence of LPS from Escherichia coli O111:B4 (100 ng/mL; Sigma-Aldrich). After 22 hours of culture at 37°C and 5% CO2, supernatant was harvested and stored at −80°C for subsequent cytokine/chemokine quantitation.

Flow Cytometry
Freshly isolated PBMCs were surface stained as previously described [27, 28]. Cells were acquired using a Canto flow cytometer (BD Biosciences), and analysis was performed with FlowJo software, version 8.5.3 (Tree Star). At least 400 000 events were acquired in a large gate including lymphocytes and monocytes, and analysis was performed with FlowJo software, version 8.5.3 (Tree Star). At least 400 000 events were acquired in a large gate including lymphocytes and monocytes, and mDCs were defined as lineage (CD16, CD14, CD3, and CD20)–negative HLA-DR+CD123+CD11c+ cells. Gated mDCs were analyzed for PD-L1, PD-L2, and costimulatory molecule (CD40, CD80, and CD86) expression. Monocytes were analyzed within the monocyte gate (cells with high forward/side-scatter characteristics) and further subdivided according to CD14 and CD16 expression. HLA-DR and PD-L1 expression were assessed using mean fluorescence intensities (MFIs) within each subset. Lymphocytes were analyzed within the lymphocyte gate.

Cytokine, Chemokine, Soluble CD14 (sCD14), LPS-Binding Protein (LBP), and Plasma LPS Quantification
Human Cytokine LINCOplex (Millipore) and Luminex LX100 (Luminex) kits were used to quantify the following cytokines and chemokines according to the manufacturers’ instructions: interleukin 10 (IL-10), interleukin 12 (IL-12), macrophage inflammatory protein 1β (MIP-1β), and TNF-α in culture supernatants collected as described above; and IL-10, interleukin 6 (IL-6), TNF-α, monocyte chemotactic protein 1 (MCP-1), and MIP-1β in serum obtained using centrifugation of freshly drawn blood and stored at −80°C until use. An enzyme-linked immunosorbent assay was used to quantify serum levels of sCD14 (R&D) and LBP (Hycult). Plasma LPS levels were quantified using the Limulus amebocyte assay (Cambrex) according to the manufacturer’s instructions. Plasma samples were diluted and pretreated as previously described [29]. All samples were assayed in duplicate.

Plasma Viral Load and Proviral DNA Assessment
Viremia was quantified by reverse transcription polymerase chain reaction (RT-PCR). For HIV-1, an assay by Roche was used, and the lower limit of quantification was 40 RNA copies/mL; for HIV-2, a previously described assay was used [30], and the lower limit of quantification was 200 RNA copies/mL. Cutoff values were considered for statistical analysis in cases when detection was below these levels. HIV-1 and HIV-2 total DNA was quantified using real-time PCR, amplifying conserved regions in HIV-1 and HIV-2 gag with a sensitivity of 5 copies, as previously described [15].

Statistical Analysis
Statistical analysis was conducted with Mann–Whitney U tests and Spearman correlations, using GraphPad Prism, version 5.00 (GraphPad Software). Multivariate linear regression analysis was conducted with Stata, version 12.1 (Stata). Results were expressed as medians and ranges, and P values of <.05 were considered statistically significant.

RESULTS
HIV-2 Infection Is Associated With Marked Monocyte Activation and PD-L1 Upregulation
Monocyte imbalances were investigated in untreated HIV-positive individuals (Table 1). Viremia was low to undetectable even in HIV-2–positive patients with low CD4+ T-cell counts. Although the time of seroconversion was unknown, these patients had most likely been infected for a long period, given the very slow rate of CD4+ T-cell decline that characterizes HIV-2 infection [15, 19].

Median numbers of circulating monocytes were similar in HIV-2–positive subjects (500 cells/µL; range, 216–832 cells/µL), HIV-1–positive subjects (564 cells/µL; range, 273–703 cells/µL), and age-matched seronegative subjects (489 cells/µL; range, 231–1285 cells/µL). Monocytes were further analyzed on the basis of CD14 and CD16 expression [31, 32], as illustrated in Figure 1A. We found that HIV-2–positive individuals showed a significant expansion of CD16+ cells among CD14+ monocytes, compared with seronegative subjects, and increased levels of HLA-DR in both CD16- and CD16+ monocytes, the latter featuring the highest expression of the molecule, suggesting an activated phenotype (Figure 1B) [3]. Evidence of monocyte activation occurred even in HIV-2–positive individuals without clear CD4+ T-cell depletion (ie, those with a CD4+ T-cell count of >350 cells/µL; Figure 1A and 1B). Accordingly, in contrast to HIV-1, a multivariate analysis that controlled for age and ethnicity found that the monocyte alterations observed in HIV-2–positive patients
Figure 1. Monocyte imbalances in human immunodeficiency virus type 2 (HIV-2)–positive patients. A, Representative flow cytometry analysis of monocyte subpopulations, the scatterplot refers to an HIV-2–positive individual with a CD4+ T-cell count of 571 cells/µL and undetectable levels of viremia. A gate including only monocytes (monogate) was defined by forward-scatter and side-scatter characteristics. Cells were further gated according to the expression of CD14 and CD16, and the relative frequency of CD16+ cells among total CD14bright monocytes is shown. The graph shows the proportion of the CD16+ subset among total CD14bright cells in seronegative (Seroneg) individuals, HIV type 1–positive individuals (HIV-1), and HIV-2–positive individuals. Mean fluorescence intensity (MFI) of HLA-DR (B) and PD-L1 (C) in CD16– (middle graphs) and CD16+ (right-hand graphs) monocyte subsets. Histograms on the left represent molecule expression among CD16– and CD16+ monocyte subsets of an HIV-2–positive individual with a CD4+ T-cell count of 571 cells/µL and undetectable levels of viremia. D, Soluble CD14 (sCD14) serum levels in subgroups of HIV-1–positive, HIV-2–positive, and seronegative individuals representative of the cohorts as a whole in terms of both CD4+ T-cell counts and viremia (where applicable). Each symbol represents 1 individual, and bars represent median values. Closed circles indicate individuals with a CD4+ T-cell count of <350 cells/µL. P values of <.05 were considered statistically significant and are shown for all statistically significant differences observed.
were unrelated to the levels of CD4+ T-cell depletion (Supplementary Table 1). Thus, obvious monocyte alterations occurred in HIV-2 infection before an upregulation of T-cell activation markers [18]. Of note, PD-L1 expression was also markedly upregulated in both CD14bright monocyte subsets in HIV-2–positive individuals, a feature not observed in the HIV-1 cohort (Figure 1C). These monocyte disturbances were unaffected by age and ethnicity after adjustment for CD4+ T-cell levels (Supplementary Table 1).

Additionally, circulating levels of sCD14, a biomarker of monocyte activation [33] and HIV disease progression [34, 35], were significantly higher in the HIV-2 cohort, compared with the seronegative cohort, reaching levels similar to those found in HIV-1–positive individuals (Figure 1D).

Moreover, despite the low levels of plasma HIV-2 (Table 1), the expansion of CD16+ cells among CD14bright monocytes was significantly higher in the viremic patients (Figure 2A). Accordingly, CD16+ cell expansion among CD14bright monocytes directly correlated with viremia in both HIV-2 infection (r = 0.4059, P = .0321) and HIV-1 infection (r = 0.6491, P = .0011).

Notably, it also positively correlated with levels of proviral DNA quantified in total PBMCs in HIV-2–positive individuals (r = 0.5466, P = .0047; n = 25) but not in HIV-1–positive individuals (r = 0.0973, P = .6749; n = 21).

Given the recent studies suggesting that HIV-2 is better able than HIV-1 to infect myeloid cells [21, 36, 37], we recruited 8 additional HIV–2–positive individuals with a CD4+ T-cell count of >350 cells/µL, undetectable viremia, and a profile of monocyte disturbances similar to that described above. We obtained purified monocytes by a cell-sorting procedure to quantify levels of proviral DNA. We found proviral DNA levels to be below the test cutoff (5 copies/10^6 cells) in all patients, which suggested that the documented monocyte alterations were unrelated to a significant reservoir of infected circulating monocytes.

Additionally, monocyte activation occurred during HIV-2 infection, even in the absence of detectable circulating virus. Accordingly, aviremic HIV–2–positive patients showed significantly increased expression of HLA-DR and PD-L1 in the CD16+ subset, as well as increased sCD14 levels, compared with seronegative subjects (Figure 2B–D).

**Figure 2.** Impact of human immunodeficiency virus type 2 (HIV-2) plasma viral load on monocyte imbalances. HIV-2–positive individuals and HIV type 1 (HIV-1)–positive individuals were stratified according to presence or absence of detectable viremia and were compared in terms of frequency of CD16+ cells among CD14bright monocytes (A), mean fluorescence intensities (MFIs) of HLA-DR (B) and PD-L1 (C) within the CD16+ subset, and serum levels of soluble CD14 (sCD14; D). Each symbol represents 1 individual, and bars represent median values. P values of < .05 were considered statistically significant and are shown for all statistically significant differences observed. Abbreviation: Seroneg, seronegative controls.
In contrast to HIV-2–positive patients, who were largely aviremic irrespective of disease stage, only 4 HIV-1–positive individuals were able to control viral replication in the absence of antiretroviral therapy [26]. These 4 “elite controllers” had undetectable viremia for 2–10 years (median, 82 months; range, 21–184 months) and relatively well-preserved CD4+ T-cell counts (median, 743 cells/μL; range, 344–1425 cells/μL). Notably, they lacked significant monocyte imbalances (Figure 2).

No significant expansion of CD14dimCD16+ cells was documented in either infected cohort (Supplementary Figure 1A). Conversely, both HIV-infected cohorts featured an expansion of CD14dimCD16− cells (Supplementary Figure 1B), whose origin and function remain debatable [38, 39].

Overall, HIV-2 infection was characterized by monocyte activation and upregulation of inhibitory molecules throughout the disease course.

Evidence Against a Major Role of Plasma LPS Levels in Monocyte Activation in HIV-2 Infection Before Significant CD4+ T-Cell Loss

Circulating LPS and other bacterial products arising from increased microbial translocation in the gut during HIV infection have been suggested to drive generalized immune activation and, particularly, monocyte activation in HIV/AIDS [29, 40]. We found no statistically significant differences in plasma LPS levels between HIV-2–positive individuals and seronegative individuals (Figure 3A) and no associations with monocyte imbalances, including levels of PD-L1 expression (P > 0.05). Conversely, patients with advanced-stage HIV-1 infection had significantly higher LPS levels than patients who were seronegative (Figure 3A), although no significant correlations were found between plasma LPS levels and CD4+ T-cell depletion or immune activation (P > 0.05).

We also quantified serum LBP, whose production is known to be induced by LPS [29]. LBP levels were similar across all cohorts (Figure 3B) and showed no correlations with any of the aforementioned parameters (P > 0.05).

Monocytes are known to become refractory upon repeated LPS stimulation [41]. Therefore, we compared the ex vivo ability of PBMCs to respond to LPS. Similarly increased IL-10, TNF-α, IL-12p40, and MIP-1β production was observed upon LPS stimulation in the 3 cohorts (Figure 3C). Moreover, there were no associations between cytokine production and markers of disease progression, except for the IL-10 stimulation index, which directly correlated with CD4+ T-cell count among HIV-2–positive subjects (r = 0.5286, P = 0.0138; n = 21) and HIV-1–positive subjects (r = 0.5250, P = 0.0445; n = 15). Of note, no relationship was found between the ability to produce cytokines upon LPS stimulation in vitro and plasma LPS level (P > 0.05).

In summary, plasma LPS did not appear to make a significant contribution to the monocyte imbalances documented here for HIV-2–positive patients.

No Major Disturbances in Serum Cytokine and Chemokine Levels in HIV-2 Disease Before Significant CD4+ T-Cell Loss

We next assessed serum levels of several cytokines and chemokines known to be produced by activated monocytes: TNF-α, MIP-1β, MCP-1, IL-6, and IL-10. HIV-2–positive patients with a CD4+ T-cell count of >350 cells/μL had serum TNF-α levels similar to those of seronegative subjects, in contrast to HIV-1–positive patients, who had a significant increase (Figure 4). At this stage of HIV-2 disease, no significant alterations were found in serum levels of IL-6, IL-10, and MIP-1β, although MCP-1 levels were significantly lower than those for seronegative subjects (Figure 4). Importantly, there were no significant correlations between the levels of plasma LPS and levels of the assessed cytokines and chemokines (P > 0.05).

Overall, we showed that the heightened monocyte activation documented in HIV-2 infection before significant CD4+ T-cell depletion did not translate into major imbalances in circulating levels of cytokines and chemokines typically produced by monocytes.

Similar Alterations in Circulating mDCs in HIV-2 and HIV-1 Infections

Monocytes differentiate into mDCs in vitro and in vivo [9, 42]. There are no data on circulating mDCs during HIV-2 infection. Thus, it is plausible that monocyte overactivation could impact on mDCs.

Circulating mDCs were assessed as illustrated in Figure 5A. The profile of mDC disturbances in HIV-2–positive patients was very similar to that found in HIV-1–positive patients (Figure 5B). Significant mDC loss, compared with that for seronegative subjects, was only observed in advanced disease stages or in the presence of detectable viremia, despite the >1 log difference in plasma viral load in the 2 infections (Figure 5B). Additionally, a significant negative correlation was observed between mDC level and the amount of circulating virus in HIV-2–positive patients (r = -0.5388, P = 0.0031) and HIV-1–positive patients (r = -0.7073, P = 0.0002). Moreover, direct correlations were found between circulating mDCs and the degree of CD4+ T-cell depletion and T-cell activation in both infected cohorts (Figure 5C), emphasizing the link between immune activation and mDC disturbances. Notably, no associations of mDC depletion with monocyte activation markers were found (Figure 5D). Similar results were obtained for mDC frequencies (data not shown).

We then asked whether mDC maturation status was altered in HIV-2 infection. Circulating mDCs expressing CD40 and/or PD-L2 were almost absent in all cohorts (data not shown). Figure 6 shows the levels of CD80, CD86, and PD-L1 in both infected cohorts, stratified according to CD4+ T-cell count and presence of detectable viremia. A marked upregulation of CD86 and PD-L1 was found in HIV-2–positive individuals with a CD4+ T-cell count of >350 cells/μL. At more advanced disease stages, both...
infected cohorts showed a significant increase in levels of the 3 molecules, compared with levels in seronegative subjects, and significant direct correlations between T-cell activation markers and expression of both CD80 and CD86 (Supplementary Table 2). Of note, no correlation between plasma LPS and mDC levels and phenotype was seen in any cohort \( (P > 0.05) \).

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**Figure 3.** Plasma levels of lipopolysaccharide (LPS) during human immunodeficiency virus type 2 (HIV-2) infection. Plasma levels of LPS (A) and serum levels of LPS-binding protein (LBP; B) in seronegative (Seroneg) individuals, HIV type 1 (HIV-1)–positive individuals, and HIV-2–positive individuals. The infected cohorts were analyzed as a whole (graphs on the left), and stratified according to the degree of CD4+ T-cell depletion (CD4+ T-cell count, >350 and <350 cells/µL; middle) or levels of viremia (undetectable vs detectable; right). C, Levels of interleukin 10 (IL-10), interleukin 12p40 (IL-12p40), tumor necrosis factor α (TNF-α), and macrophage inflammatory protein 1β (MIP-1β) in supernatants of freshly isolated peripheral blood mononuclear cells cultured with LPS (closed circles) or medium alone (open circles) for 22 hours. Each symbol represents 1 individual, and bars represent median values. \( P \) values of < 0.05 were considered statistically significant and are shown for all statistically significant differences observed.
Figure 4. Serum cytokines and chemokines in human immunodeficiency virus type 2 (HIV-2)–positive patients. Serum levels of tumor necrosis factor α (TNF-α), macrophage inflammatory protein 1β (MIP-1β), interleukin 6 (IL-6), monocyte chemotactic protein 1 (MCP-1), and interleukin 10 (IL-10) in subgroups of HIV type 1 (HIV-1)–positive individuals, HIV-2–positive individuals, and seronegative (Seroneg) individuals representative of the cohorts as a whole in respect to both CD4+ T-cell counts and viremia (where applicable). Infected cohorts were analyzed as a whole (left column), and stratified according to the degree of CD4+ T-cell depletion (middle column) or undetectable/detectable levels of viremia (right column). Each symbol represents 1 individual, and bars represent median values. P values of <.05 were considered statistically significant and are shown for all statistically significant differences observed.
Figure 5. Circulating myeloid dendritic cells (mDCs) in human immunodeficiency virus type 2 (HIV-2)–positive patients. A, Representative flow cytometric analysis of mDCs performed after successive gates on lymphocytes and monocytes defined according to forward-scatter and side-scatter characteristics, as well as HLA-DR+ cells that do not express lineage markers (left scatterplot); and CD123-CD11c+ cells (right scatterplot). Numbers represent the percentage of cells within the illustrated gates from an HIV-2–positive patient with a CD4+ T-cell count of 523 cells/µL and undetectable levels of viremia. The percentage of mDCs among peripheral blood mononuclear cells was 0.25%, which corresponded to 7 mDCs/µL. B, Absolute numbers of circulating mDCs in HIV type 1 (HIV-1)–positive individuals, HIV-2–positive individuals, and seronegative (Seroneg) individuals (left). The infected cohorts were stratified according to the degree of CD4+ T-cell depletion (middle) or viremia status (right), and mDC levels were compared. Each symbol represents 1 individual, and bars represent median values. P values of <.05 were considered statistically significant and are shown for all statistically significant differences observed. C, Correlation between absolute numbers of circulating mDCs and percentages of CD4+ T-cell count (left), HLA-DR+ cells among CD4+ T cells (middle), and HLA-DR+CD38+ cells among CD8+ T cells (right). P values of <.05 were considered statistically significant and are shown in bold. D, Correlation between absolute numbers of circulating mDCs and frequency of CD16+ monocytes among CD14bright cells (left) and soluble CD14 (sCD14) serum levels (right).
Figure 6. Expression of costimulatory and inhibitory molecules in circulating myeloid dendritic cells (mDCs) in human immunodeficiency virus type 2 (HIV-2) infection. Graphs show the percentage of CD80⁺, CD86⁺, and PD-L1⁺ cells among mDCs, as well as the ratio of the PD-L1 mean fluorescence intensity (MFI) and CD86 MFI among mDCs, in HIV type 1 (HIV-1)-positive individuals, HIV-2-positive individuals, and seronegative (Seroneg) individuals (left). Infected cohorts were stratified according to CD4⁺ T-cell counts (middle) or viremia status (right). Each symbol represents 1 individual, and bars represent median values. P values of < .05 were considered statistically significant and are shown for all statistically significant differences observed.
Interestingly, the balance between inhibitory and costimulatory molecules among mDCs, as assessed by the PD-L1 MFI/CD86 MFI ratio, was remarkably distinct in the 2 infections. This ratio was unaltered in HIV-2–positive individuals irrespective of disease stage, further supporting a role for the PD-1/PD-L1 pathway in the relatively “benign” course of HIV-2 infection. Conversely, a significant reduction of this ratio was observed in HIV-1–positive individuals with advanced disease, compared with HIV-2–positive counterparts and seronegative individuals (Figure 6).

In summary, monocyte disturbances in HIV-2 disease before marked CD4+ T-cell depletion did not seem to translate into major mDC alterations. Nevertheless, HIV-2 disease progression was associated with progressive mDC loss accompanied by a more differentiated phenotype remarkably similar to that observed during HIV-1 infection.

**DISCUSSION**

To our knowledge, this is the first comprehensive study of monocytes and mDCs in HIV-2–positive individuals, and we found a state of heightened monocyte activation and progressive mDC depletion in this natural model of attenuated HIV disease. We found (1) significant expansion of CD14bright CD16+ monocytes, accompanied by upregulation of HLA-DR expression and increased levels of sCD14; (2) monocyte activation, even in HIV-2–positive individuals with undetectable viremia and a CD4+ T-cell count of >350 cells/µL; (3) lack of association of monocyte imbalances with plasma LPS or LBP levels, and no impairment in their ability to respond to LPS upon in vitro stimulation; (4) overexpression of the inhibitory molecules PD-1/PD-L1 in myeloid cells that may help limit the increase in serum levels of proinflammatory cytokines and chemokines usually produced by activated monocytes; and (5) progressive decline in the level of circulating mDCs and acquisition of a more differentiated mDC phenotype, despite the reduced viremia that characterizes HIV-2 infection.

Our observation of marked PD-L1 upregulation in HIV-2–positive individuals, even in the absence of detectable viremia and/or marked CD4+ T-cell loss, suggests that, in HIV-2 infection, the PD-1/PD-L1 pathway may help limit immune activation throughout the disease course. In agreement with this suggestion, HIV-2–positive patients had a significantly higher ratio of inhibitory (PD-L1) and costimulatory (CD86) molecules on mDCs, compared with HIV-1–positive patients, implying that the overall balance of signaling is tipped toward suppression, thereby possibly slowing down the increase in immune activation. This was further supported by the lack of increase in serum levels of monocyte-produced proinflammatory cytokines and chemokines in HIV-2–positive individuals. Our observations are in agreement with recently published data suggesting that, during HIV-2 disease, there is a strict control of PD-1/PD-L1 expression on T cells, which is disrupted in the context of HIV-1 infection, that possibly helps limit immunopathology and restrain disease progression [43]. Additionally, our previous data on plasmacytoid dendritic cells (pDCs) during HIV-2 disease also showed significantly increased levels of PD-L1 expression, irrespective of disease stage [27]. Of note, this was not observed in the few HIV-1 “elite controllers” included in this study. This should be investigated in larger cohorts of these particular HIV-1–positive individuals, who, similar to HIV-2–positive patients, have low levels of viremia in the absence of antiretroviral receipt. Taken together, these data suggest that generalized PD-L1 overexpression may constitute a specific “HIV-2 signature.”

Of note, we found that mDC alterations during HIV-2 infection were clearly similar to those found in HIV-1–positive individuals paired for the degree of CD4+ T-cell depletion, despite the distinct viremia and disease course. It has been suggested that disturbances in cell trafficking may contribute to a preferential loss of circulating mDCs in HIV-1 infection, although the limited data available on lymphoid tissues from HIV-2–positive individuals precluded the evaluation of this possibility [44, 45]. Our finding of a direct association between T-cell activation and mDC disturbances in both HIV-1 and HIV-2 infections supports a major contribution of persistent immune activation.

The heightened monocyte and mDC activation could be partly related to the proposed ability of HIV-2 to better counteract host restriction factors that limit HIV-1 replication in myeloid cells [37, 46]. Recent data suggest that SAMHD1 is a main player in this restriction [21, 36, 47]. This molecule is targeted by Vpx, a viral protein only encoded by HIV-2 and some simian immunodeficiency virus (SIV) strains but not by HIV-1 [48]. Thus, HIV-2 would be expected to circumvent SAMHD1-imposed restriction to viral replication in monocytes. However, we found no detectable proviral load in monocytes isolated from HIV-2–positive individuals with a CD4+ T-cell count of >350 cells/µL, suggesting that, while Vpx could facilitate monocyte infection by HIV-2, additional restriction pathways may operate upon HIV-2 infection in these cells. Although these results argue against a significant amount of productive HIV-2 replication in monocytes, it is possible that viral entry per se impacts on monocyte activation, a possibility that warrants further investigation. mDC depletion could also be ascribed to direct virus infection, as suggested by recent studies addressing the role of Vpx [21, 36]. Although this possibility was not addressed in our study, previous data have shown mDCs to be less susceptible to HIV-2 than HIV-1 infection in vitro [20].

Our data also suggest that factors other than direct viral infection are implicated in mDC decline, as we found similar decreases in mDC levels in HIV-2–positive individuals and HIV-1–positive individuals, despite their distinct levels of viremia.
Another factor that could be related to the observed monocyte and mDC activation is circulating LPS levels, which have been described to correlate with HIV disease severity \[29, 40\]. However, we found no association between monocyte and mDC disturbances and circulating LPS in HIV-2 infection. These findings argue against a major role for microbial translocation, assessed as circulating LPS, in HIV-2 infection, as reported for some African cohorts \[49\]. Of note, even though our HIV-2 cohort was enriched in African individuals, no associations were found between ethnicity and LPS levels that could impact on our results (data not shown). A recent report has suggested that, in the context of HIV-1 infection, microbial translocation may upregulate PD-1 levels on monocytes, leading to increased IL-10 production upon PD-L1 binding, with consequent attenuation of immune responses \[50\]. Importantly, we found no associations between PD-1 expression on monocytes and plasma LPS levels or serum IL-10 \( (P > 0.05) \), although a significant increased in mean PD-1 levels \( (\pm \text{SEM [Standard Error of the Mean]}) \) among total monocytes was observed in HIV-2-positive patients \( (1998 \pm 216) \), both in comparison with HIV-1-positive individuals \( (1493 \pm 92; P = 0.0272) \) and with seronegative individuals \( (1405 \pm 167; P = 0.0174) \), in direct correlation with PD-L1 expression \( (r = 0.6251; P = 0.0004) \).

In conclusion, to our knowledge we are the first to investigate monocyte and mDC activation during HIV-2 infection, a naturally occurring form of attenuated HIV disease. We found evidence of monocyte activation throughout HIV-2 disease, as well as progressive circulating mDC depletion, with upregulation of activation markers, tightly correlated with markers of T-cell activation. Our data emphasize the relationship between generalized immune activation and myeloid cell disturbances in HIV-2–positive individuals, albeit with no apparent link to circulating LPS levels. Moreover, monocytes and mDCs featured marked upregulation of the inhibitory molecule PD-L1 from early on in HIV-2 infection, possibly helping limit activation-related immunopathology and resulting in a more “benign” disease course. These findings challenge current paradigms concerning the role of monocytes and mDCs in HIV/AIDS and have the potential to identify novel strategies to modulate inflammatory states.

### Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

### Notes

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**R. C. and R. T. designed and performed experiments, analyzed data, and wrote the manuscript; R. B. F., R. S. S., A. P. B., and P. G. performed experiments; E. V. selected patients and discussed results; R. M. M. V. contributed to the design of the study and data interpretation; and A. E. S. designed the research, analyzed data, and wrote the manuscript.**

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