Immunosuppressive Tryptophan Catabolism and Gut Mucosal Dysfunction Following Early HIV Infection

Mohammad-Ali Jenabian,1,2,a Mohamed El-Far,3 Kishanda Vyboh,1,2 Ido Kema,11 Cecilia T. Costiniuk,1,3 Rejean Thomas,6 Jean-Guy Baril,2 Roger LeBlanc,1,8 Cynthia Kanagaratham,2 Danuta Radzioch,2 Ossama Allam,9,10 Ali Ahmad,9,10 Bertrand Lebouché,1 Cécile Tremblay,5,9 Petronela Ancuta,5,9 and Jean-Pierre Routy1,2,4; for the Montreal Primary infection and Slow Progressor Study Groups

1Chronic Viral Illnesses Service, 2Research Institute, 3Division of Infectious Diseases, 4Division of Hematology, McGill University Health Centre, 5CHUM Research Centre, 6Clinique Médicale l’Actuel, 7Clinique Médicale Quartier Latin, 8Clinique Médicale OPUS, 9Department of Microbiology and Immunology, 10CHU Ste-Justine Research Center, University of Montreal, Quebec, Canada; and 11Department of Laboratory Medicine, University Medical Center, University of Groningen, The Netherlands

Background. Tryptophan (Trp) catabolism into kynurenine (Kyn) contributes to immune dysfunction in chronic human immunodeficiency virus (HIV) infection. To better define the relationship between Trp catabolism, inflammation, gut mucosal dysfunction, and the role of early antiretroviral therapy (ART), we prospectively assessed patients early after they acquired HIV.

Methods. Forty patients in the early phase of infection were longitudinally followed for 12 months after receiving a diagnosis of HIV infection; 24 were untreated, and 16 were receiving ART. Kyn/Trp ratio, regulatory T-cells (Tregs) frequency, T-cell activation, dendritic cell counts, and plasma levels of gut mucosal dysfunction markers intestinal-type fatty acid–binding protein, soluble suppression of tumorigenicity 2, and lipopolysaccharide were assessed.

Results. Compared with healthy subjects, patients in the early phase of infection presented with elevated Kyn/Trp ratios, which further increased in untreated patients but normalized in ART recipients. Accordingly, in untreated subjects, the elevated Treg frequency observed at baseline continued to increase over time. The highest CD8+ T-cell activation was observed during the early phase of infection and decreased in untreated patients, whereas activation normalized in ART recipients. The Kyn/Trp ratio was positively associated with CD8+ T-cell activation and levels of inflammatory cytokines (interleukin 6, interferon-γ-inducible protein 10, interleukin 18, and tumor necrosis factor α) and negatively associated with dendritic cell frequencies at baseline and in untreated patients. However, ART did not normalize plasma levels of gut mucosal dysfunction markers.

Conclusions. Early initiation of ART normalized enhanced Trp catabolism and immune activation but did not improve plasma levels of gut mucosal dysfunction markers.

Keywords. indoleamine 2,3-dioxygenase-1 (IDO-1); tryptophan; HIV early infection; ART; regulatory T-cells (Tregs); inflammation; dendritic cells; gut mucosal dysfunction; microbial translocation; sST2.

The early phase of human immunodeficiency virus (HIV) infection is characterized by rapid depletion of the total CD4+ T-cell pool and a dramatic increase in immune activation associated with a high plasma HIV load [1]. During the early phase of infection, massive CD4+ T-cell depletion is observed mainly in gut-associated lymphoid tissues (GALTs), impairing mucosal integrity and resulting in progressive microbial translocation from the gut to the periphery [2, 3]. Microbial translocation has been recognized as a major contributor to immune dysfunction and persistent immune...
activation during HIV infection [1–3]. Moreover, this persistent immune activation is independently associated with a greater risk of non-AIDS-related morbidity and mortality [2].

We and others have reported that tryptophan (Trp) catabolism into kynurenine (Kyn) by indoleamine 2,3-dioxygenase-1 (IDO-1), expressed by dendritic cells (DCs) and monocytes, skews CD4+ T-cell differentiation into regulatory T cells (Tregs) instead of T-helper (Th17) cells and directly impairs T-cell responses [4, 5]. It is well recognized that IDO-1–induced Treg production is associated with immunosuppressive effect during pregnancy, cancer, and viral infections [6, 7]. In HIV infection, the altered Th17/Treg balance is directly linked to increased and persistent IDO-1 activity via interferon γ signaling and Toll-like receptor stimulation [4], and Trp breakdown is associated with immune activation [8]. Indeed, increased IDO-1 activity is associated with the degree of microbial translocation and HIV disease progression and predicts mortality [4, 5, 9]. More recently, it has been reported that dysbiosis of the gut microbiota is associated with IDO-1 and contributes to HIV disease progression [10]. However, the causal effects of Trp catabolism and gut mucosal dysfunction on the inflammatory response during the early phase of HIV infection have not yet been addressed.

Initiation of antiretroviral therapy (ART) during the early phase of infection results in a lower HIV burden and a reduced HIV reservoir size [11–14]. Early initiation of ART has also been associated with reduction of anti-HIV antibody formation [15] and has a significant impact on health-related quality of life [16]. Receipt of ART for 6 months reduces Trp catabolism, although not to normal levels [17]. We and others have previously shown that long-term ART reduces both IDO-1 expression and normalizes IDO-induced Trp catabolism [4, 5, 18]. However, limited data are available regarding the influence of early ART on immunosuppressive IDO-1 activity and recovery of gut mucosal dysfunction.

In this study, we compared IDO-induced Trp catabolism, immune activation, markers of myeloid-lymphoid inflammation, and gut mucosal dysfunction in HIV-infected adults who did and those who did not receive ART during the early phase of infection.

MATERIAL AND METHODS

Study Population
Peripheral blood mononuclear cells and plasma specimens were longitudinally collected from HIV-infected individuals for whom HIV acquisition was estimated to have occurred <180 days earlier; all were participants in the Montreal HIV primary infection study. Diagnosis of HIV infection was established on the basis of positivity for p24 antigen and/or a detectable HIV RNA load, subsequently confirmed by Western blot. Some patients started ART during the first year of infection, based on their CD4+ T-cell counts and the decision of the physicians and patients. Samples were obtained from 12 elite controllers in the Canadian Slow Progressor Cohort and from 12 healthy controls at the Chronic Viral Illness Service, McGill University Health Centre (MUHC; Montreal, Canada; Table 1).

Ethics Statement
This study was conducted according to the Declaration of Helsinki and received approval from the MUHC Ethical Review Board. All study subjects provided written informed consent for participation in the study.

Measurement of the IDO-1 Enzymatic Activity
Plasma levels of Trp and its catabolite, Kyn, were measured by an automated, online, solid-phase-extraction high-performance liquid chromatography–tandem mass spectrometry method, as we previously reported, and IDO-1 enzymatic activity was determined by calculating the Kyn/Trp ratio [5, 19].

Flow Cytometry
Flow cytometry was performed by a 4-laser LSRII flow cytometer (BD Bioscience, Mississauga, Canada). The following antibodies were used: CD3-Pacific blue, CD4-FITC, CD4-PerCP Cy5.5, CD4-PECy5, CD4-APC-Cy7, CD8-Alexa700, CD25-PE, CD27-Alexa700, CD28-PECy5, CD57-APC, CD127-PECy7, CD38-APC, HLA-DR-APC Cy7, CCR5-PE, α4-FTC, β7-PECy5, Lineage-FTC (including anti-CD3, CD14, CD19, CD20, and CD56), CD11c-APC, and CD123-PE (BD Bioscience); CD45RA-EC (Beckman Coulter, Mississauga, Canada); and CD8-APC Fluor 780 and FOX-P3 Alexa 488 (eBioscience, San Diego, California). The viability marker Vivid (Invitrogen, Burlington, Canada) was used to exclude dead cells from analysis. Data were analyzed using FlowJo software v7.6.5.

Multiplex Quantification of Plasma Inflammatory Markers
Plasma levels of inflammatory soluble factors interleukin 6 (IL-6), interleukin 18 (IL-18), interferon γ–induced protein 10 (IP-10), and tumor necrosis factor α (TNF-α) were measured in duplicate using a ProcartaPlex Multiplex Immunoassay according to the manufacturer’s instructions (eBioscience). Mean fluorescence intensities for each analyte in each sample were detected using the MAGPIX instrument (Luminex, Austin, Texas), and the results were analyzed using xPONENT 4.2 software (Millipore) to obtain the protein concentration of each soluble factor in each sample.

Measurement of Plasma Levels of Intestinal-Type Fatty Acid–Binding Protein (I-FABP), Soluble Suppression of Tumorigenicity 2 (sST-2), and Lipopolysaccharide (LPS)
Plasma levels of markers of I-FABP and sST-2 were measured by enzyme-linked immunosorbent assays, using commercially available kits from HyCult Biotech (Uden, the Netherlands) and R&D Systems (Minneapolis, Minnesota), respectively. Plasma levels of the marker of microbial translocation, LPS, were measured using commercially available kits from Cusabio (Wuhan, China).
Statistical Analysis
Statistical analyses were performed using GraphPad Prism software, version 5. Kruskal–Wallis tests were performed for comparisons between study groups. Unpaired t tests or Mann–Whitney U tests were used for comparisons of 2 nonpaired study variables, according to the sample size. The Wilcoxon matched pairs test was used for comparisons of paired study variables. The Spearman rank correlation test was used to identify associations among study variables.

RESULTS
Study Population
Forty-eight patients who received a diagnosis of HIV infection during the early phase of infection (ie, <180 days after acquisition) were enrolled. Clinical characteristics of patients at baseline and 1 year after follow-up are described in Table 1. The estimated dates of infection were as follows: <30 days (n = 4), 31–90 days (n = 10), and 91–180 days (n = 34). Twenty-four patients remained untreated after 1 year of follow-up. Seventeen patients received ART during the first year of infection, including 7 within 3 months, 5 between 3 and 6 months, and 5 between 6 and 12 months following the estimated infection date. One patient, whose viral load remained elevated despite ART, was excluded from longitudinal analysis. Samples from 7 patients were not available for longitudinal assessment at the 12-month follow-up time point. Therefore, 40 patients (24 untreated patients and 16 ART recipients) were assessed longitudinally. Compared with baseline values, ART significantly improved the mean number (±standard deviation [SD]) of CD4+ T cells (626 ± 205 vs 456 ± 192 cells/mL; P = .003) and yielded a mean viral load below the level of detection (<1.7 vs 6.3 ± 6.8 log10 copies/mL; P = .0005; data not shown).

Early ART Initiation Rapidly Normalized Immunosuppressive Trp Catabolism and Halted Treg Expansion
Short-term ART can reduce Trp catabolism, although not to normal levels [17]. We previously reported that Trp catabolism was normalized to levels similar to those in healthy subjects after an 8-year mean duration of successful ART [5]. Here, we assessed Trp catabolism following ART initiation during the early phase of infection. Patients in the early phase of infection displayed an elevated mean plasma level of Kyn (±SD), compared with healthy subjects (2.58 ± 0.67 vs 1.86 ± 0.52 μmol/L; Figure 1A). Recipients of early ART had a decreased mean level of Kyn (±SD), whereas the level in untreated patients remained high (2.05 ± 0.50 vs 2.62 ± 0.60 μmol/L). Consistently, the mean Kyn/Trp ratio (±SD) was elevated at baseline, normalized in ART recipients, and remained high in untreated patients, compared with healthy subjects (0.05 ± 0.02, 0.04 ± 0.01, 0.05 ± 0.02, and 0.04 ± 0.01, respectively; Figure 1B). Mean Kyn levels (±SD) decreased following ART initiation, compared with baseline (2.77 ± 0.71 vs 2.06 ± 0.51 μmol/L; Figure 1C), and further increased over time for those who remained untreated.
Accordingly, the Kyn/Trp ratio followed a similar trend, as it decreased longitudinally following ART initiation and increased when patients remained untreated (Figure 1D and 1F). These results demonstrate that Trp catabolism rapidly normalized following early ART initiation and continued to increase in untreated patients.

As Trp catabolism by IDO-1 contributes to the expansion of Tregs in cancer and HIV infection [4–6], we evaluated the peripheral frequency of Tregs following early infection. At baseline, the mean frequency (±SD) of CD4⁺CD25⁺CD127⁺ Tregs during the early phase of infection was similar to that in healthy subjects (data not shown), while it significantly increased over time during the longitudinal follow-up in untreated patients (6.22% ± 1.53% vs 5.6% ± 1%; P = .01) but not in ART recipients (6.22 ± 1.6 vs 5.7 ± 1.46; P = .14), compared with baseline (data not shown). When transcription factor FoxP3 was included in the analysis, the expression of
CD4<sup>+</sup>CD25<sup>high</sup>CD127<sup>low</sup>FoxP3<sup>high</sup> Tregs also did not differ between subjects in the early phase of infection and healthy subjects (Figure 2A). However, we observed a significant increase in the frequency of FoxP3<sup>+</sup>Tregs in untreated individuals but not in ART recipients, compared with healthy subjects (Figure 2A). When assessed longitudinally, an increasing trend in the proportion of FoxP3<sup>+</sup>Tregs was observed in untreated patients, but the proportion remained unchanged following ART initiation (Figure 2B and 2C). These results indicate that early ART administration can break down the progressive expansion of Tregs ultimately observed during the chronic phase of infection in untreated patients.

**Kyn/Trp Ratio Is Associated With Generalized Immune Activation**

As HIV infection is associated with persistent immune activation, we first evaluated CD8<sup>+</sup> T-cell activation by measuring CD38/HLA-DR coexpression. Patients at baseline displayed the most elevated mean frequency (±SD) of activated CD8<sup>+</sup> T cells, compared with any other group, which normalized rapidly following ART initiation (17.40% ± 13.86% vs 2.64% ± 1.89%; Figure 3A). Mean CD8<sup>+</sup> T-cell activation (±SD) was lower during the chronic phase of infection in untreated patients, compared with baseline, although activation continued to remain elevated as compared to that in healthy subjects (10.40% ± 7.40% vs 1.95% ± 1.66%; Figure 3A). Longitudinal assessment revealed a drastic reduction in mean CD8<sup>+</sup> T-cell activation (±SD) from baseline in ART recipients (22.41% ± 17.99% vs 2.64% ± 1.89%; Figure 3B), whereas there was a slight decrease in CD8<sup>+</sup> T-cell activation in untreated patients (14.70% ± 9.37% vs 10.40% ± 7.40%; Figure 3C). Interestingly, positive correlations were observed between the Kyn/Trp ratio and CD8<sup>+</sup> T-cell immune activation at baseline and for untreated patients (Figure 3D and 3E) but not in ART recipients (data not shown). Consistent with our previous studies [5], a strong correlation was observed between viral load and the Kyn/Trp ratio at baseline (P = .017 and R = 0.34) and in untreated patients (P = .02 and R = 0.47; data not shown).
Indoleamine 2,3-dioxygenase-1 (IDO-1) enzymatic activity was associated with CD8$^+$ T-cell immune activation and the ratio of CD8$^+$ T cells to CD4$^+$ T cells (CD8$^+$/CD4$^+$ T-cell count ratio) in both early and acute phases of human immunodeficiency virus infection. A, Cross-sectional comparison of the coexpression of immune activation markers HLA-DR and CD38 on CD8$^+$ T cells within study groups. Boxes denote median values and interquartile ranges, and whiskers denote the largest and smallest values. B and C, Change in CD8$^+$ T-cell immune activation between the early phase of infection and after antiretroviral therapy (ART) initiation (B) or during the chronic phase of infection, without treatment (C). D and E, Positive correlation between CD8$^+$ T-cell activation and the ratio of kynurenine (Kyn) to tryptophan (Trp), a marker of IDO-1 enzymatic activity, in the early phase of infection (D) and in untreated patients (E). F and G, Correlation between CD8$^+$/CD28$^-$CD57$^+$ frequency and the Kyn/Trp ratio in the early phase of infection (F) and in untreated patients (G). H and I, Negative correlation between the CD8$^+$/CD4$^+$ T-cell count ratio and the Kyn/Trp ratio in the early phase of infection (H) and in untreated patients (I).
HIV disease progression markers IL-6 and IP-10 and the Kynurenine (Kyn) to Tryptophan (Trp) ratio was positively associated with the Kyn/Trp ratio. In our cohort, we observed an increase in the Kyn/Trp ratio during chronic infection for untreated patients (Figure 3).

Frequencies of senescent cells, defined as CD8\(^+\)CD28-CD57\(^+\) cells, are notably increased in chronic HIV infection as a marker of immune dysfunction [20]. We therefore assessed their levels in association with Kyn/Trp ratio. The mean frequency of senescent cells (±SD) was elevated at baseline, compared with that in healthy subjects (34.35% ± 9.90% vs 12.80% ± 12.37%), and treatment status did not affect senescence expression within study groups (data not shown). When assessed longitudinally, no significant difference between baseline and after ART initiation was observed (mean frequency (±SD), 36.33% ± 9.36% vs 35.90 ± 12.36%), indicating a halt in further increases in senescence, although untreated patients displayed a marked increase (33.17% ± 10.23 vs 38.20% ± 11.19; \(P = .02\), data not shown). Importantly, a strong positive correlation was only observed between the frequency of senescent cells and the Kyn/Trp ratio during chronic infection for untreated patients (Figure 3). A lower ratio of CD4\(^+\) T cells to CD8\(^+\) T cells (hereafter, the “CD4\(^+/\)CD8\(^+\) T-cell count ratio”) in HIV-infected patients is related to both innate and adaptive immune activation and is associated with a higher risk of non-AIDS-related events even with ART [21]. In our cohort, we observed an increase in the mean CD4\(^+/\)CD8\(^+\) T-cell count ratio (±SD) in ART recipients (0.97 ± 0.39 vs 0.58 ± 0.28; \(P = .0009\)) but not untreated subjects (0.82 ± 0.63 vs 0.85 ± 0.57; \(P = .2\)), compared with baseline (data not shown). Importantly, we observed a negative correlation between the CD4\(^+/\)CD8\(^+\) T-cell count ratio and the Kyn/Trp ratio at baseline and in untreated subjects (Figure 3H and 3I). Furthermore, the Kyn/Trp ratio was positively associated with the HIV disease progression markers IL-6 and IP-10 and the inflammatory cytokines IL-18 and TNF-\(\alpha\) (Table 2). Collectively, the results indicate that Trp catabolism is associated with generalized immune activation, immunosenescence, and HIV-mediated inflammation, which could revert back to normal following early ART initiation.

A Lower Circulating DC Frequency Is Associated With a Higher Kyn/Trp Ratio

As DCs constitute the main cell types that express IDO-1, we evaluated the changes in DC frequency following the early phase of infection in relation to Trp catabolism. At key study visits, no differences were observed between the frequency of CD11c\(^+\) myeloid DCs (mDCs) and CD123\(^+\) plasmacytoid DCs (pDCs) at baseline and for untreated patients, compared with healthy subjects (data not shown). However, comparison of patients to themselves over time revealed a significant increase in mean frequencies (±SD) of peripheral mDCs (0.31% ± 0.13% vs 0.50% ± 0.17%) and pDCs (0.15% ± 0.1% vs 0.22% ± 0.12%) following early ART initiation, compared with baseline (Figure 4A and 4B), while frequencies remaining unchanged in untreated patients (0.4% ± 0.19% vs 0.41% ± 0.18% for mDCs and 0.2% ± 0.1% vs 0.18 ± 0.1% for pDCs; Figure 4C and 4D). These results suggest that inflammation observed at baseline and in untreated patients might contribute to the migration of DCs into inflammatory sites, such as the gut mucosa, resulting in a decrease in their relative frequency in the peripheral [22]. Importantly, we observed that a lower peripheral DC frequency was negatively associated with the Kyn/Trp ratio at baseline and in untreated patients (Figure 4E–H). This is in

Table 2. Inflammatory Cytokine Changes Following Early Antiretroviral Therapy (ART) Initiation in Association With the Ratio of Kynurenine (Kyn) to Tryptophan (Trp)

<table>
<thead>
<tr>
<th>Inflammatory Cytokine</th>
<th>Patients With Early HIV Infection</th>
<th>HIV-Infected ART Recipients</th>
<th>HIV-Infected Untreated Subjects</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma Level, pg/mL</td>
<td>Correlation With Kyn/Trp Ratio</td>
<td>Plasma Level, pg/mL</td>
<td>Correlation With Kyn/Trp Ratio</td>
</tr>
<tr>
<td>IL-6</td>
<td>Mean ± SD</td>
<td>(P = .06; R = 0.3463)</td>
<td>0.6 ± 0.65</td>
<td>NS</td>
</tr>
<tr>
<td>IL-18</td>
<td>Mean ± SD</td>
<td>(P = .03; R = 0.32)</td>
<td>158 ± 185</td>
<td>NS</td>
</tr>
<tr>
<td>IP-10</td>
<td>Mean ± SD</td>
<td>(P &lt; .0001; R = 0.60)</td>
<td>155 ± 85</td>
<td>NS</td>
</tr>
<tr>
<td>TNF-(\alpha)</td>
<td>Mean ± SD</td>
<td>NS</td>
<td>35 ± 21</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>7–145</td>
<td>7–79</td>
<td>7–152</td>
</tr>
</tbody>
</table>

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; IL-6, interleukin 6; IL-10, interleukin 10; IL-18, interleukin 18; IP-10, interferon \(\gamma\)-induced protein 10; NA, not applicable; NS, not significant; SD, standard deviation; TNF-\(\alpha\), tumor necrosis factor \(\alpha\).
Figure 4. Lower circulating dendritic cell (DC) frequency is associated with a higher ratio of kynurenine (Kyn) to tryptophan (Trp), a marker of IDO-1 enzymatic activity, following primary human immunodeficiency virus infection. A and B, Frequency of myeloid dendritic cells (mDCs; A) and plasmacytoid DCs (pDCs; B) in a longitudinal analysis of patients in the early phase of infection and then following antiretroviral therapy (ART) initiation. C and D, Frequency of mDCs (C) and pDCs (D) in a longitudinal analysis of patients in the early phase of infection and then in the chronic phase of infection, without treatment. E and F, Negative correlations between the peripheral frequency of mDCs (E) and pDCs (F) in patients in the early phase of infection. G and H, Negative correlations between the peripheral frequency of mDCs (G) and pDCs (H) and the Kyn/Trp ratio in untreated patients. Abbreviation: PBMC, peripheral blood mononuclear cell.
line with higher expression of IDO-expressing DCs in the gut mucosa and higher dietary Trp catabolism observed in experimental intestinal inflammation [23].

Absence of Change in the Frequency of T Cells Expressing α4β7 Gut-Homing Receptor Following Early ART Initiation

It has been shown that the loss of circulating CD4⁺ T cells expressing the gut-homing marker α4β7 integrin mirrors the CD4⁺ T-cell depletion in the GALT and occurs within days after simian immunodeficiency virus infection [24]. In all study groups, the mean frequency (±SD) of CD4⁺ T-cell subsets expressing α4β7 was lower than that for CD8⁺ T cells (patients in the primary phase of infection: 9.2% ± 3.3% vs 20.67% ± 8.8%; ART recipients: 9.37% ± 3.6% vs 24% ± 9.8%; and untreated subjects: 9% ± 3.2% vs 18.35% ± 7.5%; P < .0001 for all comparisons), and no correlation was observed between the viral load or Kyn/Trp ratio and the α4β7⁺ T-cell frequency (data not shown). These α4β7⁺ T cells expressed higher mean levels (±SD) of CCR5, compared with total CD4⁺ T cells (patients in the early phase of infection: 12.85% ± 7.6% vs 6.9% ± 4.3% [P < .0001]; ART recipients: 6.03% ± 4% vs 4.46% ± 2.5% [P = .02]; and untreated subjects: 10.3% ± 7.1% vs 6% ± 4.4% [P < .0001]; data not shown). A significant mean decrease (±SD) in CCR5⁺α4β7⁺ and memory α4β7⁺CD4⁺ T-cell expression in both untreated subjects (13% ± 8.7% vs 10.3% ± 7.1% [P = .03] and 92.2% ± 4.2% vs 90.3% ± 6.1% [P = .02], respectively) and ART recipients (90% ± 5.6% vs 86.1% ± 8.7% [P = .002] and 12.6% ± 6% vs 6 ± 3.9%, respectively [P = .002]), compared with baseline, was observed longitudinally, which suggests that the preferential depletion of α4β7⁺ cells results in damage to gut mucosal immunity (data not shown).

Early Treatment With ART Did Not Improve Markers of Gut Mucosal Damages and Microbial Translocation

Massive CD4⁺ T-cell depletion in the GALT causes impaired mucosal integrity, resulting in microbial translocation [2, 3]. We therefore evaluated the changes in plasma markers associated with gut mucosal damage, including I-FABP [25], sST-2 [26–28], and the marker of microbial translocation, LPS [29]. Our results showed an increase in plasma levels of both I-FABP and sST-2 at baseline, compared with levels in healthy subjects, which remained high even in patients receiving ART.

Figure 5. Increase in plasma markers of gut mucosal damage and microbial translocation activity following primary human immunodeficiency virus infection. A cross-sectional analysis within all study groups of the levels of plasma markers of intestinal mucosal damage, intestinal-type fatty acid-binding protein (I-FABP; A) and soluble suppression of tumorigenicity 2 (sST-2; B), and a marker of microbial translocation, lipopolysaccharide (LPS; C). Boxes denote median values and interquartile ranges, and whiskers denote the largest and smallest values. Abbreviation: ART, antiretroviral therapy.
DISCUSSION

We previously reported that Trp catabolism into Kyn, in association with HIV load and lower CD4^+ T-cell count, is immunosuppressive in HIV infection via alteration of the Th17/Treg balance [5, 19]. The Kyn/Trp ratio is also recognized as an independent predictor of HIV disease progression and mortality [21, 30]. In this longitudinal assessment of patients beginning the early phase of infection, we observed that early ART initiation had a beneficial impact by normalizing Trp catabolism to levels observed in healthy subjects and decreasing levels of various markers of myeloid and lymphoid inflammation. Several studies of Austrian, Ugandan, and Chinese cohorts of HIV-infected patients showed that 6–12 months of successful ART can reduce Kyn/Trp ratios by only 50% [17, 18, 31]. In contrast with these studies, we observed a rapid normalization of the Kyn/Trp ratio by early ART following the early phase of infection, indicating the importance influence of the timing of ART initiation on Trp catabolism [31].

Because we and others have shown that IDO-induced Trp catabolism contributes to the generation of Tregs in HIV-infected patients [4, 5, 19], we evaluated the frequency of peripheral Tregs. In relation to the Kyn/Trp ratio, we observed a continuous increase in the Treg frequency in untreated patients, compared with baseline values and the frequency in healthy subjects. Importantly, this increase was halted by early ART initiation. Our results showed that the Kyn/Trp ratio was associated with immune activation and levels of the HIV disease progression markers IL-6 and IP-10 (CXCL10) [32, 33] and the inflammatory cytokines IL-18 and TNF-α. Another enzyme, tryptophan 2,3-dioxygenase (TDO), mainly expressed by the liver, is also able to catabolize Trp [34]. However, liver TDO activity is suppressed when extrahepatic IDO-1 activity is induced during inflammation [34].

Correlation between the Kyn/Trp ratio and immune activation in our study suggests that IDO-1 is the key player in Trp catabolism in our setting. We showed that early ART initiation can rapidly reduce immune activation in association with a decreased Kyn/Trp ratio. A persistently low CD4/CD8^+ T-cell count ratio during virologically suppressive ART is associated with increased innate and adaptive immune activation, immunosenescence, Kyn/Trp ratio, and higher risk of morbidity/mortality [21]. It has been shown that patients who started ART early, within 6 months of infection onset, had a greater CD4/CD8^+ T-cell count ratio increase than patients who started ART >2 years after infection onset [21]. Interestingly, we showed that early ART initiation improved the CD4^+/CD8^+ T-cell count ratio and prevented emergence of the CD8^+ T-cell senescence population, in line with the decreased Kyn/Trp ratio.

DCs are one of the main IDO-expressing cell types. We observed that lower frequencies of peripheral mDCs and pDCs were negatively associated with higher IDO-1 enzymatic activity. In HIV infection, DCs migrate from peripheral blood to the GALT, where they contribute to immune activation [22]. Furthermore, the frequency of IDO-expressing DCs in gut mucosa results in a higher immunosuppressive catabolism of dietary Trp observed in intestinal inflammation [23]. Therefore, our results indicate that early ART initiation could contribute to lower mucosal inflammation, thereby resulting in a higher frequency of peripheral DCs and a decrease in IDO-induced catabolism of dietary Trp in the gut.

Disruption of gut mucosal integrity mediates persistent immune activation in HIV infection [1–3, 35]. In acute infection, gut memory CD4^+ T cells expressing CCR5 are preferentially infected and depleted, with 60% of CD4^+ T cells being lost within 2–3 weeks of HIV infection [35–37]. Indeed, loss of mucosal CD4^+ T cells expressing the gut-homing marker α4β7 occurs within a few days of simian immunodeficiency virus infection [24]. We observed decreases in CCR5^+ and memory α4β7^+ T cells in both untreated subjects and ART recipients, compared with baseline, which favor preferential depletion of α4β7 cells, resulting in reduced gut mucosal immunity. However, early ART initiation improved recovery of circulating α4β7^+ T cells.

Limited T-cell access to interleukin 7 in GALT due to fibrosis and architectural distortion of mucosa represents a major limit of T-cell reconstitution [3, 38]. Fibrogenesis in gut mucosa starts during the early phase of infection, owing to HIV and cytokine-mediated immune activation (eg, interleukin 6, transforming growth factor β, and hyaluronic acid) and contributes to collagen deposition, increased mucosal permeability, and microbial translocation [1–3, 9, 35, 38]. Indeed, gut epithelial dysfunction associated with the Kyn/Trp ratio and innate immune activation are independent predictors of mortality in ART recipients [9]. We therefore assessed the changes in plasma markers on gut mucosal injury following early ART initiation. I-FABP, a cytosolic enterocyte protein [39], is a marker of enterocyte damage [9, 40, 41], and its level strongly correlates with mortality in HIV-infected patients [9]. We also evaluated plasma levels of sST-2, a recently identified marker of gut mucosal damage. ST-2 is the receptor for interleukin 33 from the interleukin 1 family, which is involved in proinflammatory reactions and Th2 immune responses [42]. Inflammatory sST2 [27, 43] binds with interleukin 33 to sequestrate its effects and has been recently described as a biomarker of intestinal inflammatory
disorders [26, 28]. Our results showed an early increase in plasma levels of 1-FABP and sST-2 during the early phase of infection, which remained high even in patients receiving early ART. Accordingly, an increase in LPS plasma levels was observed at baseline, which remained high in the chronic phase of infection, even in ART recipients. LPS stimulates monocyte differentiation into DCs and is a microbial product and a marker of microbial translocation [3, 44, 45]. The stable increase in levels of sST-2 and LPS at all stages of infection, despite early ART initiation, indicates that gut mucosal damage starts in the early phase of infection and results in microbial translocation that cannot be rapidly repaired by ART. Further studies are needed to assess the possible role of early ART initiation at longer follow-up time points.

Our observations are consistent with prior studies showing that ART initiation in primary infection leads to partial rather than complete reconstitution of gut mucosa [37, 46, 47]. To reconcile the persistent gut mucosal damage and microbial translocation with rapid normalization of the Kyn/Trp ratio and immune activation by early ART initiation, we propose the “liver firewall” hypothesis. It is likely that early ART initiation leads to decreased HIV-mediated immune activation by controlling viral replication, while relatively low levels of microbial products (LPS) may not trigger systemic activation, as phagocytic Kupffer cells in the liver are at work [48, 49]. Indeed, the liver serves as a firewall to filter gut microbial products that have penetrated systemic vascular circuits, and Kupffer cell exhaustion may only occur late in HIV infection or in cirrhotic patients [3]. The mechanism of incomplete GALT reconstitution is unclear and may be due either to viral or immune-mediated accelerated T-cell destruction or potentially to alterations in T-cell homing to the gut [37]. Combinations of immunotherapies with ART could be beneficial, as our group has shown that administration of recombinant interleukin 7 in chronically infected ART recipients with low CD4+ T-cell count recovery results in partial gut mucosal recovery [50].

Collectively, our findings show that early ART initiation has a beneficial influence on HIV patients by normalizing enhanced Trp catabolism and decreasing various markers of myeloid and lymphoid inflammation without impacting gut mucosal dysfunction.

**Notes**

**Acknowledgments.** Subjects in the early phase of infection were screened, recruited, and followed up by the following physicians, whom we thank for their collaboration: Drs B. Trottier, S. Vézina, L. Charest, M. Milne, E. Huchet, S. Lavoie, J. Friedman, M. Duchastel, and F. Villela, at l’Actuel Medical Clinic; P. Coté, M. Potter, B. Lessard, M. A. Charron, S. Dufresne, and M. E. Turgeon, at Quartier Latin Medical Clinic; Drs D. Rouleau, L. Labrecque, C. Fortin, A de Pokomandy, B. Trottier, V. Hal-Gagné, M. Munique, B. Deligne, and V. Martel-Laferrère, at UHRESS CHUM-Hôtel-Dieu and Notre-Dame; and N. Gilmore, M. Fletcher, and J. Szabo, at MUHC Chest Institute. We are thankful for their collaboration. We thank Ms A. F. Vassal and M. Legault, for administrative support; V. Lafontaine, for laboratory processing and shipment; members of the Network Lab Spec Committee, for overall approval of the project; Mrs Angie Massicotte, for her clerical assistance and coordination; Ms Stephanie Matte, from the Canadian HIV-1 Slow Progressor cohort, and Byhan Pine-da, from the MUHC, for coordination and blood banking; Jacqui Sas and Jim Pankovich, from the CIHR Canadian HIV Trials Network, for study implementation and coordination; and Dr Dominique Gauchat and Annie Gosselin, from the flow cytometry core of the CHUM-Research Centre, Saint-Luc Hospital, Montréal, Canada, for technical assistance.

**Financial support.** This work was supported by the Canadian Institutes of Health Research (grants MOP 103230 and CTN 257), the Canadian Foundation for AIDS Research (CANFAR; grant 023-512), Fonds de la Recherche Québec-Santé (FRQ-S): Thérapie cellulaire et Réseau SIDA/Maladies infectieuses, Canadian HIV cure enterprise (CanCure), the CANFAR/Canadian HIV Trials Network (postdoctoral fellowship to M.-A. J.), Canadian HIV Trials Network (postdoctoral fellowship to B. L.) and McGill University (Louis Lowenstein Chair in Hematology and Oncology to J.-P. R.).

**Potential conflicts of interest.** All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**


