Increased Tryptophan Catabolism Is Associated With Increased Frequency of CD161$^+$Tc17/MAIT Cells and Lower CD4$^+$ T-Cell Count in HIV-1 Infected Patients on cART After 2 Years of Follow-Up

Julie C. Gaardbo, MD, PhD,† Marius Trøsied, MD, PhD,‡§ Birgitte Stiksrud, MD,‡‖ Øivind Midttun, PhD,¶ Per M. Ueland, MD, DMSc, Henrik Ullum, MD, PhD,† and Susanne D. Nielsen, MD, DMSc**

Background: HIV infection is associated with increased ratio between kynurenine and tryptophan (KTR) in plasma, increased microbial translocation, expansion of regulatory T cells (Tregs), and depletion of Tc17/mucosa-associated invariant T (MAIT) cells. The association between these parameters and the impact of KTR on CD4$^+$ T-cell recovery in HIV-infected patients on combination antiretroviral therapy (cART) after 2 years of follow-up was investigated.

Methods: Forty-one HIV-infected individuals treated with cART for a minimum of 2 years were included. Tregs, CD161$^+$Tc17/MAIT cells, naive cells, immune activation, senescence, and apoptosis were measured using flow cytometry. Soluble CD14 (sCD14), lipopolysaccharide, and tryptophan metabolites in plasma were measured retrospectively before cART and at inclusion initiation using Limulus Amebocyte Lysate colometric assay, enzyme-linked immunosorbent assay, and tandem mass spectrometry, respectively. KTR was calculated, and patients were divided into 2 groups defined by high vs. low KTR. CD4$^+$ T-cell count was determined at inclusion and after 2 years of follow-up.

Results: KTR decreased after cART initiation. Patients on cART with high KTR displayed an immunological profile with high sCD14, high percentage Tregs, low percentage CD161$^+$Tc17/MAIT cells, low percentage naive cells, low CD4/CD8 ratio, and poor immune reconstitution after 2 years of follow-up compared with patients with low KTR.

Conclusions: Our results support the hypothesis that tryptophan catabolism, indoleamine 2,3-dioxygenase 1 (IDO1) activation, microbial translocation, and perturbed distribution of Tregs and CD161$^+$Tc17/MAIT cells are part of a vicious circle that perpetuates exhaustion of the immune system and progression of untreated HIV infection and challenge immune reconstitution in patients on cART.

Key Words: tryptophan catabolism, kynurenine pathway, microbial translocation, regulatory T cells, Tc17 cells, MAIT cells

(*J Acquir Immune Defic Syndr* 2015;70:228–235)

INTRODUCTION

In HIV infection, CD4$^+$ T cells are depleted, and excess activation of the immune system, in particular chronic activation of T cells, is a well-documented contributor to HIV disease progression.[1–8] Chronic immune activation may be a result of severe depletion of CD4$^+$ T cells including interleukin 17 (IL-17)–producing T cells (Th17 cells) within the gut during acute infection, leading to a leaky mucosal barrier.[9,10] This allows transfer of microbial remnants such as lipopolysaccharide (LPS) from the gut to enter the circulation, a phenomenon denoted microbial translocation.[11,12] LPS triggers inflammation and immune activation in part by stimulating toll-like receptor 4 (TLR4) on monocytes and other innate immune cells. The soluble form of CD14 (sCD14) plays an important role for signaling by transferring LPS to the TLR4 complex.

Another hallmark of progressive HIV infection is increased percentage of anti-inflammatory regulatory T cells (Tregs) found in untreated and treated patients.[13,14–16] Microbial translocation and chronic immune activation may contribute to this increased percentage of Tregs.[17,18]

Lately, a link between tryptophan metabolism, microbial translocation, and Tregs has been suggested. Tryptophan

Received for publication August 18, 2014; accepted June 9, 2015.

From the *Viro-immunology Research Group, Department of Infectious Diseases, Rigshospitalet, University Hospital of Copenhagen, Copenhagen, Denmark; †Department of Clinical Immunology, Rigshospitalet, University Hospital of Copenhagen, Copenhagen, Denmark; ‡Department of Infectious Diseases, Oslo University Hospital, Oslo, Norway; §KG Jebsen Centre of Inflammation Research, University of Oslo, Oslo, Norway; ‖Institute of Clinical Medicine, University of Oslo, Oslo, Norway; and ¶Bevital A/S, Bergen, Norway.

Supported by The Novo Nordisk Foundation; Augustinus Foundation; Rigshospitalet; Aage and Johanne Louis-Hansen Foundation, University of Copenhagen; KG Jebsen Centre of Inflammation Research, University of Oslo; and Department of Infectious Diseases, Oslo University Hospital.

The authors have no conflicts of interest to disclose.

J.C.G. designed the study, included the patients, performed the laboratory analyses, analyzed the data, and wrote the article; M.T. designed the study, performed the laboratory analyses, and critically revised the article; B.S. contributed to figure design and critically revised the article; P.M.U. and Ø.M. performed the laboratory analyses and critically revised the article; H.U. designed the study and critically revised the article; and S.D. N. was principal investigator, designed the study, and critically revised the article.

Correspondence to: Susanne D. Nielsen, MD, DMSc, Department of Infectious Diseases M8632, Rigshospitalet, Blegdamsvej 9B, 2100 Copenhagen, Denmark (e-mail: sdn@dadlnet.dk).

is an essential amino acid being metabolized primarily through the kynurenine pathway. Indoleamine 2,3-dioxygenase 1 (IDO1) is the rate-limiting enzyme for catabolism of tryptophan through the kynurenine pathway.[19] Kynurenine is subsequently metabolized to a number of metabolites, including the neurotoxic metabolites 3-hydroxykynurenine and quinolinic acid. The ratio between plasma concentrations of kynurenine and tryptophan (KTR) is used as an indirect measure of IDO activity.[20] Increased circulating levels of kynurenine pathway metabolites have been reported to be associated with Alzheimer disease, Huntington disease, schizophrenia, cancer, and HIV-associated neurocognitive disorders.[21–24] These findings may be explained by an increased catabolism of tryptophan being associated with suppression of T-cell responses, potentially due to decreased tryptophan levels per se and to elevated levels of the metabolites.[19] In HIV infection, reduced tryptophan levels in progressive disease were acknowledged more than a decade ago.[25] In animal models, the regulatory function of T cells has been shown to depend on the ability to induce IDO expression by dendritic cells.[19] Furthermore, in humans, it has been shown that IDO activates Tregs and blocks their conversion into $CD4^+$Th17-like cells[26] and in HIV infection, an altered balance of $CD4^+$Th17 cells and Tregs by IDO has been reported.[20]

Importantly, combination antiretroviral therapy (cART) only partially restores the damaged mucosal barrier, and IL-17–producing $CD4^+$Th17 cells are depleted in HIV infection despite successful cART.[11,27,28] Also, $CD8^+$ IL-17–producing cells, named Tc17 cells or mucosa-associated invariant T (MAIT) cells, have been suggested to play an important role in mucosal defense. In contrast to $CD4^+$Th17 cells, $CD8^+$Tc17/MAIT cells are considered to be a part of the innate immune system, important for mucosal integrity, and involved in the antibacterial and antifungal defense.[29] Recently, we and others have shown that both the total number and relative frequency of Tc17/MAIT cells are reduced in treated and untreated HIV infection.[28,30] To date, no studies have explored a potential association between tryptophan catabolism and Tc17/MAIT cells. We hypothesized that increased tryptophan catabolism could be associated with depletion of $CD161^+$Tc17/MAIT cells, expansion of Tregs, and reduced $CD4^+$ T-cell count in patients receiving cART (Fig. 1A).

METHODS

Forty-one HIV-infected patients on cART with sustained viral suppression were included in this study. All patients had been on cART for a minimum of 2 years and had CD4 nadir <250 cells per microliter. Exclusion criteria were hepatitis B and C, malignant disease, and pregnancy. These patients also participated in another study, where they were grouped according to $CD4^+$ T-cell count as published elsewhere.[31] However, as plasma samples were only available for analyses in 41/77 patients, the cohort was

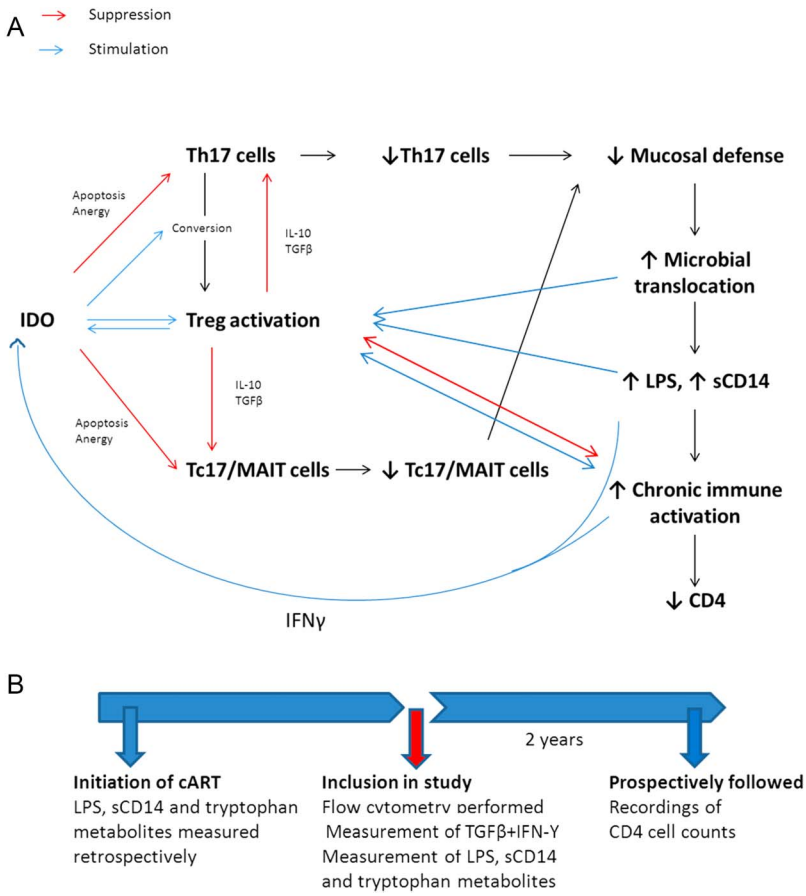

FIGURE 1. A, Figure showing the overall hypothesis of the study. Increased tryptophan catabolism could be associated with depletion of Tc17/MAIT cells, expansion of Tregs, and reduced CD4 T-cell count in patients receiving cART. B, Flow cytometry and measurement of TGF-β and IFN-γ, LPS, sCD14, and tryptophan metabolites were performed in samples from the time of inclusion. LPS, sCD14, and tryptophan metabolites were also measured in plasma samples collected from the patients before initiation of cART (n = 37). CD4/$CD8^+$ T-cell counts were measured before cART initiation and at the time of inclusion. Furthermore, $CD4^+$ T-cell counts were recorded prospectively and until 2 years of follow-up.

considered one group in this study. Clinical characteristics are presented in Table 1.

Flow cytometry and measurement of TGF-β and IFN-γ, LPS, sCD14, and tryptophan metabolites were performed in samples from the time of inclusion. LPS, sCD14, and tryptophan metabolites were also measured in plasma samples collected from the patients before initiation of cART (n = 37). CD4/CD8$^+$ T-cell counts were measured before cART initiation and at the time of inclusion. Furthermore, CD4$^+$ T-cell counts were recorded prospectively and until 2 years of follow-up (Fig. 1B). Data on Tc17 cells, Tregs, and immune activation have previously been published.[28,31]

The study was performed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and approved by the local ethical committee (H-2-2009-089 and H-4-2010-012) and the Danish Data Protection Agency.

Blood Analysis

EDTA-stabilized blood was used to obtain a full blood count, for flow cytometry (except for Tregs) and measurements of tryptophan with metabolites, sCD14, and LPS. Heparinized blood was used to isolate peripheral blood mononuclear cells by means of density gradient centrifugation. Fresh isolated peripheral blood mononuclear cells were used for determination of Tregs.

Flow Cytometry

CD3 was used in combination with CD4 or CD8 to identify CD4$^+$ and CD8$^+$ T cells, respectively. Naive (CD27$^+$CD45RA$^+$CCR7+), recently activated (CD69$^+$) and chronically activated (CD38$^+$HLA-DR+), senescent (CD28$^-$CD57$^+$), and apoptotic (CD28$^-$CD95$^+$) T cells were determined. Furthermore, CD161$^+$Tc17/MAIT cells (CD3$^+$CD8$^+$CD161^{high}) and Tregs (CD4$^+$CD25$^+$CD127^{low}-Foxp3+) were determined. Erythrocytes were lysed with lysing solution (Becton Dickinson [BD], Franklin Lakes, NJ), and samples were resuspended in FACSFlow (BD). Gating strategies have been published elsewhere.[31,32] In brief, 100 μL of fresh EDTA blood was incubated with fluorescent dye–conjugated monoclonal antibodies according to the manufacturer's instructions. Monoclonal antibodies used to determine lymphocyte subsets were isotype control IgG1/IgG2a phycoerythrin (PE), IgG1 peridinin chlorophyll proteins–cyanine (PerCP-Cy5.5), IgG1/IgM fluorescein isothiocyanate (FITC), IgG1/IgG2b allophycocyanin (APC), IgG1 PE-Cy7, IgG1 APC-H7, CCR7-PE, CD161-PE, CD28-PE, Foxp3-PE, CD8-PerCP-Cy5.5, CD25-PerCP-Cy5.5, CD3-FITC, CD127-FITC, CD57-FITC, HLA-DR-APC, CD45RA-APC, CD95-FITC, CD27-PE-Cy7, CD38-PE-Cy7, CD69-PE-Cy7, and CD4-APC-H7, all purchased from BD. Six-color acquisition was performed using FACSCanto, and data were processed using FACSDiva software (BD). For each sample, a minimum of 50,000 cells were acquired.

TABLE 1. Clinical Characteristics of the Study Population at Inclusion in the Study

	Low KT Ratio (n = 20)	High KT Ratio (n = 21)	*P*
Gender (male), n (%)	18 (90)	17 (81)	0.663
Ethnicity (white), n (%)	18 (90)	21 (100)	0.232
Age, yrs	45 (38–55)	56 (45–66)	0.019
CD4 nadir, cells/μL	105 (69–141)	98 (66–130)	0.766
CD4$^+$ T-cell count, cells/μL	461 (367–554)	348 (255–442)	0.083
CD8$^+$ T-cell count, cells/μL	695 (560–829)	903 (708–1099)	0.077
CD4/CD8 ratio	0.71 (0.56–0.86)	0.40 (0.33–0.52)	0.001
Treatment duration, mo	52 (38–67)	78 (49–106)	0.110

Values are given as median (IQR).
IQR, interquartile range; KT, kynurenine–tryptophan.

LPS and sCD14

LPS was analyzed by Limulus Amebocyte Lysate colometric assay (Lonza, Walkersville, MD) according to the manufacturer's instructions, with the following modifications: samples were diluted 10-fold to avoid interference with background color and preheated to 68°C for 12 minutes before analyses to dissolve immune complexes. sCD14 (R&D Systems, Minneapolis, MN) was analyzed by ELISA according to the manufacturer's instructions, and samples were diluted 300-fold. Samples for both analyses were measured in duplicates.

Kynurenine Pathway

Tryptophan and metabolites were measured using liquid chromatography–tandem mass spectrometry (LC-MS/MS; Agilent and Applied Biosystems/MSD Sciex) as described earlier.[33] KTR was calculated by dividing the plasma concentration of kynurenine (in nanomoles per liter) by the concentration of tryptophan (in micromoles per liter) and subsequently multiplying by 1000.

Analysis of TGF-β and IFN-γ

To determine the ex vivo production of TGF-β and IFN-γ, 0.4 mL of heparinized peripheral blood was cultured in 1.6 mL RPMI 1640 and PHA. The culture was incubated at 37°C for 24 hours after which the supernatant was collected and stored at −80°C until use. TGF-β and IFN-γ levels were measured in the supernatants by the Flourokine Human MultiAnalyte Profiling Base Kit assay (R&D Systems) in duplicate according to the manufacturer's instructions and analyzed on Luminex 100 platform (Luminex Corp., Austin, TX).

Statistical Analyses

Results are given as mean values with 95% confidence intervals, unless otherwise stated. Data on patients before and after initiation of cART were analyzed using paired *t* tests. Results of KTR both before and after cART were divided into 2 groups with low/high KTR, respectively, according to the median. Comparison between groups with low vs. high KTR was evaluated using unpaired *t* test. Associations were calculated using univariate linear regression. Because of non-normal distribution, log-transformation was applied on sCD14. The data were thereafter normally distributed.

Two-tailed P values <0.05 were considered significant. Statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA).

RESULTS

A total of 41 patients were included. Figure 1B demonstrates the timeline of when study participants were included in relation to data collection.

Concentrations of Tryptophan, Kynurenine, and KTR

Levels of tryptophan in patients increased from 46.6 μM/L (41.4–51.9) before cART to 61.2 μM/L (57.0–65.3) after cART ($P < 0.001$). In contrast, levels of kynurenine decreased from 2.5 μM/L (2.2–2.8) to 1.8 μM/L (1.6–2.0, $P < 0.001$). KTR decreased from 67.7 (48.3) to 30.9 (27.3–34.4) after initiation of cART ($P < 0.001$, Fig. 2A–C).

Median KTR before cART was 35.8 (29.4–40.9) in the group with low KTR vs. 75.0 (66.1–111.2) in the group with high KTR. Median KTR after cART was 23.9 (19.2–25.3) in the group with low KTR vs. 36.1 (32.4–40.5) in the group with high KTR.

KTR, sCD14, and LPS in Patients Initiating cART

Likewise, the level of sCD14 decreased after initiation of cART [668 (559–778) vs. 525 (458–592) ng/mL, $P = 0.044$], whereas no difference in LPS levels before and after initiation of cART was found [123 (107–139) vs. 114 (105–122) pg/mL, $P = 0.251$].

Before initiation of cART, patients with high KTR also had higher levels of sCD14 compared with those with low KTR [856 (687–1025) vs. 504 (400–609) ng/mL, $P < 0.001$], whereas no difference was found in LPS levels [116 (99–134)

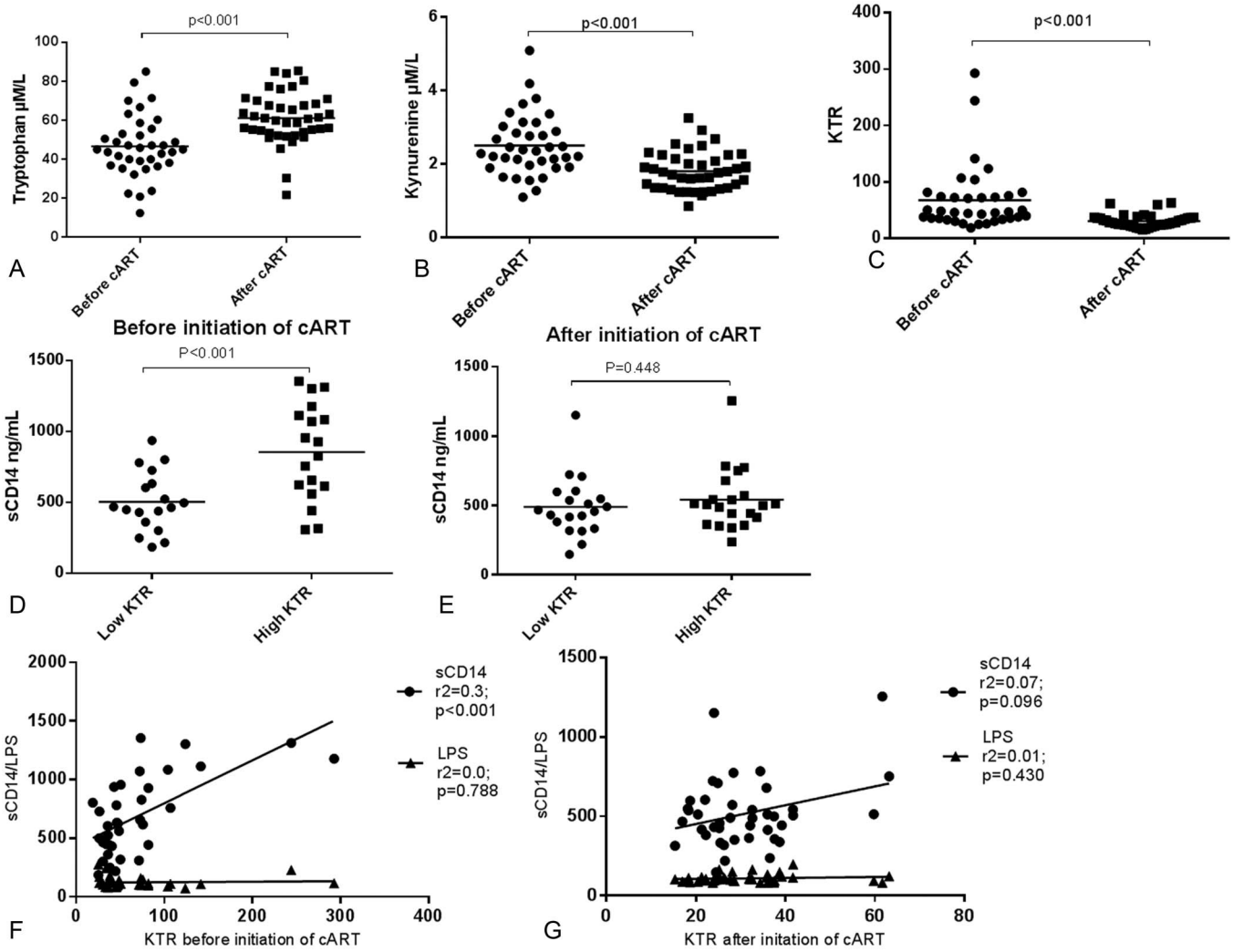

FIGURE 2. A–C, Concentrations of tryptophan (A), kynurenine (B), and KTR (C) in HIV-infected patients before and after initiation of cART. D and E, Concentration of sCD14 in patients with high vs. low KTR before (D) and after initiation of cART (E). F and G, Association between KTR and sCD14/LPS before (F) and after cART (G).

vs. 130 (98–162) pg/mL, $P = 0.421$]. Furthermore, KTR was positively associated with sCD14, but not LPS ($r^2 = 0.30$, $P < 0.001$ and $r^2 = 0.00$, $P = 0.789$, respectively). Finally, KTR was associated with HIV RNA before initiation of cART, but not with CD4$^+$ T-cell count ($r^2 = 0.19$, $P = 0.009$ and $r^2 = 0.06$, $P = 0.139$, respectively).

In contrast, no difference was found neither in sCD14 nor in LPS levels between patients with high vs. low KTR on cART ($P = 0.448$ and $P = 0.210$, respectively). Also, in these virally suppressed patients, the association between KTR and sCD14 was attenuated ($r^2 = 0.07$, $P = 0.096$), whereas KTR stayed unrelated to LPS ($r^2 = 0.02$, $P = 0.430$) (Fig. 2D–G).

KTR Is Positively Associated With Tregs and Negatively Associated With CD161$^+$Tc17/MAIT Cells in Patients on cART

In patients with high KTR, the percentage of Tregs was higher compared with patients with low KTR [8.2% (6.5–9.9) vs. 6.2% (5.3–7.2) of CD4$^+$ cells, $P = 0.049$, Fig. 3A]. No difference was found in the count of absolute Tregs between patients with high KTR and those with low KTR [25.3 (19.9–30.7) vs 26.2 cells/μL (21.5–30.8), $P = 0.801$]. A positive association was found between KTR and percentage Tregs of CD4$^+$ T cells as well as the concentration of TGF-β ($r^2 = 0.13$, $P = 0.021$ (Fig. 3B) and $r^2 = 0.15$, $P = 0.017$, respectively). Finally, the percentage of Tregs was positively associated with the concentration of kynurenine, but not with tryptophan ($r^2 = 0.15$, $P = 0.011$ and $r^2 = 0.02$, $P = 0.42$, respectively).

In contrast, the percentage of CD161$^+$Tc17/MAIT cells was almost 3-fold lower in patients with high KTR compared with patients with low KTR [1.1% (0.5–1.7) vs. 3.2% (1.7–4.7) of CD8$^+$ cells, $P = 0.009$, Fig. 3C]. Also, the absolute CD161$^+$Tc17 cell count seemed to be lower in patients with high KTR compared with those with low KTR, although this did not reach statistical significance [10.6 (3.6–17.7) vs. 19.6 cells/μL (9.6–29.5), $P = 0.132$]. Accordingly, this was accompanied by a negative association between KTR and percentage CD161$^+$Tc17/MAIT cells of CD8$^+$ T cells

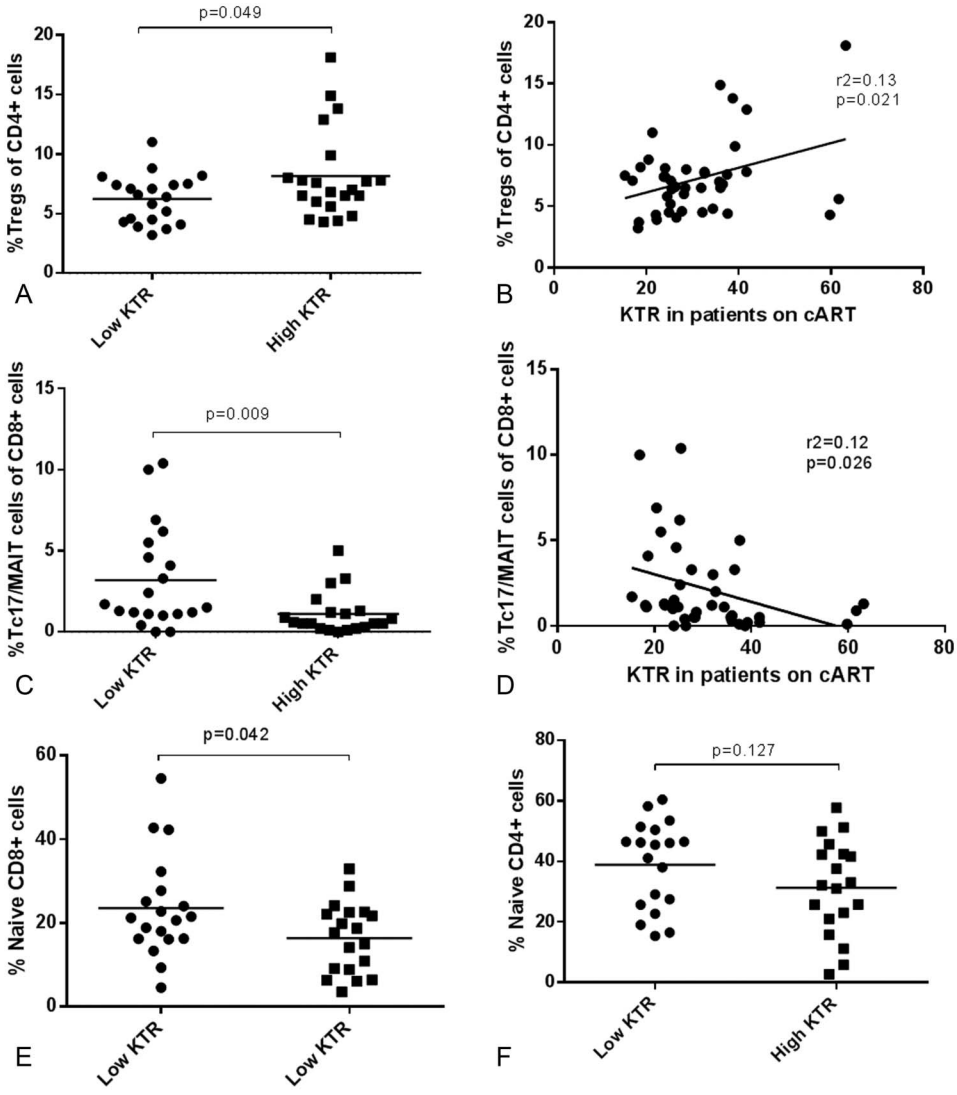

FIGURE 3. A and B, Percentage Tregs of CD4$^+$ T cells in patients with high vs. low KTR (A) and association between KTR and percentage Tregs of CD4$^+$ T cells in HIV-infected patients on cART (B). C and D, Percentage Tc17/MAIT cells of CD8$^+$ T cells in patients with high vs. low KTR (C) and association between KTR and percentage Tc17/MAIT cells of CD8$^+$ cells (D). Percentage Naive CD8$^+$ cells (E) and CD4$^+$ cells (F) high vs. low KTR.

($r^2 = 0.12$, $P = 0.026$, Fig. 3D). Also, a tendency towards negative association between the percentage of CD161$^+$Tc17/MAIT cells and the concentration of kynurenine, but not tryptophan, was found ($r^2 = 0.09$, $P = 0.063$ and $r^2 = 0.04$, $P = 0.225$, respectively).

KTR is Negatively Associated With Naive Cells and Positively Associated With Immune Activation in Patients on cART

Patients with high KTR on cART displayed lower percentages of naive CD8$^+$ T cells compared with those with low KTR [23.6% (17.7–29.4) vs. 16.4% of CD8$^+$ cells (12.4–20.4), Fig. 3E]. No significant difference was found in naive CD4$^+$ cells [38.9% (32.0–45.9) vs. 31.3% of CD4$^+$ T cells (23.8–38.9), $P = 0.127$, Fig. 3F]. Negative associations were found between KTR and both naive CD4$^+$ and CD8$^+$ T cells ($r^2 = 0.13$, $P = 0.026$ and $r^2 = 0.17$, $P < 0.01$, respectively).

Positive associations between KTR and recently activated CD4$^+$CD69$^+$ cells and apoptotic CD8$^+$CD28$^-$CD95$^+$ cells were found ($r^2 = 0.11$, $P = 0.032$ and $r^2 = 0.112$, $P = 0.035$, respectively). Also, a positive tendency was found between KTR and chronically activated CD4$^+$CD38$^+$HLA-DR and CD8$^+$CD38$^+$HLA-DR as well as senescent CD8$^+$CD28$^-$CD57$^+$ cells ($r^2 = 0.09$, $P = 0.059$; $r^2 = 0.09$, $P = 0.058$; and $r^2 = 0.09$, $P = 0.06$, respectively). No associations were found between KTR and recently activated CD8$^+$CD69$^+$ and apoptotic CD4$^+$CD28$^-$CD95$^+$ cells (data not shown).

KTR, CD161$^+$Tc17/MAIT Cells, CD4$^+$/CD8$^+$ T-cell Ratio, and Immune Reconstitution

Patients on cART with high KTR presented with a lower CD4$^+$/CD8$^+$ T-cell ratio compared with patients with lower KTR, and a negative association was found [0.4 vs. 0.7, $P = 0.001$ (Fig. 4A); $r^2 = 0.15$, $P = 0.014$, respectively]. In contrast, a strong positive association between percentage Tc17/MAIT cells of CD8$^+$ T cells and CD4$^+$/CD8$^+$ T-cell ratio was found ($r^2 = 0.36$, $P < 0.001$, Fig. 4B). Likewise, percentage CD161$^+$Tc17/MAIT cells of CD8$^+$ T cells was positively associated with CD4 nadir ($r^2 = 0.17$, $P = 0.008$).

After 2 years of follow-up, CD4$^+$ T-cell counts were recorded (Fig. 1B). Patients with low KTR had a better immune reconstitution and a higher CD4$^+$ T-cell count after 2 years of follow-up compared with patients with high KTR (ΔCD4: 88 vs. 32 cells/μL, $P = 0.051$ and CD4$^+$ T-cell count: 531 vs. 381 cells/μL, $P = 0.04$, Fig. 4C–D). Likewise, negative association was found between KTR at the time of inclusion in the study and CD4$^+$ T-cell count after 2 years of follow-up ($r^2 = 0.12$, $P = 0.039$).

DISCUSSION

The main findings of this study was that increased tryptophan catabolism as measured by KTR was associated with depletion of CD161$^+$Tc17/MAIT cells, increased frequency of Tregs, and lower immune reconstitution in HIV-infected patients receiving cART.

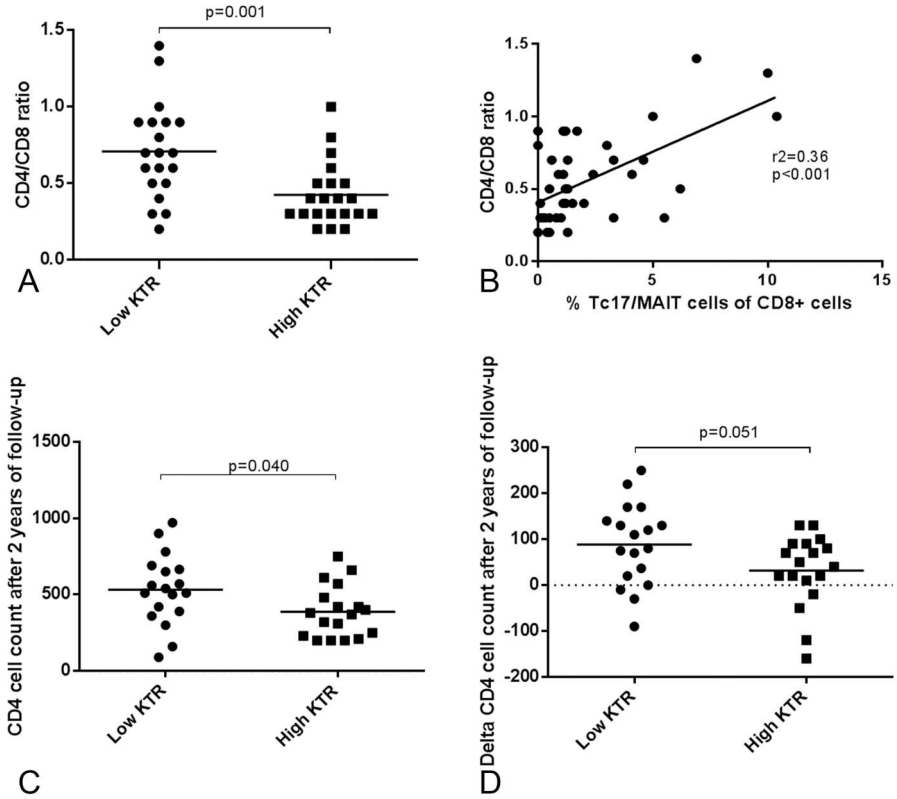

FIGURE 4. A, CD4/CD8 ratio in HIV-infected patients on cART with high vs. low KTR. B, Association between percentage of Tc17/MAIT cells of CD8$^+$ T cells and CD4/CD8 ratio in patients on cART. C and D, CD4$^+$ T-cell count (C) and delta CD4$^+$ T-cell count (D) in HIV-infected patients with high vs. low KTR after 2 years of follow-up.

Tryptophan catabolism has gained interest, as recent evidence have suggested a skewed balance of tryptophan and the downstream metabolites to be involved in a nonfavorable outcome of HIV infection. We found reduction of KTR by more than half after a median of >5 years of cART (Fig. 2C) This is in line with a recent study showing 50% reduction in KTR in Ugandans who had been on cART for 12 months[34] and with yet another study showing reduction in an American cohort treated for nearly 2 years.[35] Furthermore, despite this reduction in KTR, we found a lower CD4/CD8 ratio and a lower $CD4^+$ T-cell gain after 2 years of follow-up in patients on cART with high KTR. This finding is also in agreement with the recent report from Uganda.[34]

Apart from CD3 and CD8, Tc17/MAIT cells can be characterized by expression of $CD161^{high}$, CCR6, and IL-17.[36–39] In this study, we used CD3, CD8, and $CD161^{high}$, and this lack of IL-17 production used in the identification may influence on the results. However, it has been shown that all $CD161^{high}$ cells also express CCR6, and $CD3^+CD8^+CD161^{high}$ harbor all of the IL-17–producing $CD8^+$ cells.[36,37] Thus, $CD3^+CD8^+$ cells expressing $CD161^{high}$ identify Tc17/MAIT cells in an acceptable although not optimal way.

Apart from being proinflammatory, Tc17/MAIT cells are considered as part of the innate immune system with biological effect against bacteria and fungi.[29] A novel finding of this study is that patients with high KTR during cART had approximately 3-fold depletion in $CD161^+$Tc17/MAIT cells compared with patients with low KTR (Fig. 3C). This association may be explained by several factors working in concert as illustrated in Figure 1A. First, IDO has several immune suppressive effects, including suppression of T cells.[19] Second, Tc17/MAIT cells may be indirectly depleted by increased secretion of IFN-γ, which is known to be a strong activator of IDO.[29] Indeed, we found a negative association between $CD161^+$Tc17/MAIT cells and IFN-γ. Third, IDO can possibly deplete Tc17/MAIT cells indirectly by inducing Tregs, which secrete inhibiting enzymes such as TGF-β.[19,40] In line with other studies, we found a positive association between KTR and levels of Tregs,[20,41] strengthened by increased production of TGF-β being positively associated with KTR. Interestingly, we found positive association between Tregs and the concentration of kynurenine but not tryptophan, suggesting that excess kynurenine rather than depletion of tryptophan may lead to expansion of Tregs. However, it remains unclear whether this expansion of Tregs is beneficial or not during HIV infection.

It has been suggested that increased tryptophan degradation and IDO activity may in part be triggered by microbial translocation and monocyte activation.[20] We found that KTR was strongly associated with sCD14 in cART-naive patients, and this association was attenuated after cART initiation. In contrast to the study by Favre et al, however, we found no correlation between LPS levels and KTR in our cohort. Of note, sCD14 is shed from monocytes by several microbial antigens in addition to LPS,[42] and other products from the gut microbiota could have contributed to the association between sCD14 and KTR. Interestingly, a previous study found that a dysbiotic gut microbiota was associated with increased tryptophan catabolism and inflammation, in particular in untreated patients.[43] Thus, it is not unlikely that gut microbiota alterations could contribute to several findings in our study and should be further investigated not only in relation to tryptophan catabolism but also in relation to downregulation of Tc17/MAIT cells. This is supported by our findings of the negative relation between percentage of $CD161^+$Tc17/MAIT cells, CD4/CD8 ratio, and CD4 nadir, suggesting that depletion of $CD161^+$Tc17/MAIT cells may contribute to a nonfavorable immunological profile.

Recently, it was reported that increased tryptophan metabolism and several markers of inflammation strongly predicted all-cause mortality in patients receiving cART, whereas T-cell activation was less predictive after adjustment for $CD4^+$ T-cell count.[34] This is in line with our finding of a less favorable distribution of $CD4^+$ and $CD8^+$ T cells and impaired immune reconstitution in patients with high KTR. Furthermore, we found that those patients with high KTR displayed lower percentages of naive T cells. Also, the KTR was positively associated with immune activation, senescence, and apoptosis. This is in line with findings from in vitro studies showing that downstream metabolites of tryptophan catabolism have toxic effects on T-cell responses both suppressing proliferation and inducing cell death.[44,45] Finally, we found a positive association between KTR and HIV RNA levels before cART. These findings are in line with the positive relation of kynurenine concentrations with both the levels of $CD4^+$ and $CD8^+$ T-cell activation and HIV RNA in the cART-naive cohort by Favre et al.[20]

It has been proposed that tryptophan catabolism, IDO activation, inflammation, microbiota alterations, and microbial translocation are part of a vicious circle that perpetuates exhaustion of the immune system and progression of HIV infection.[43] In conclusion, our findings support this overall hypothesis and suggest that downregulation of $CD161^+$Tc17/MAIT cells is part of this overall picture. However, this study was limited by the lack of mechanistic investigations, a partly retrospective design, and a relative limited number of study participants. Also, gut biopsies were not available in this cohort. Indeed, it would be interesting to evaluate MAIT cells in the mucosa, as cells in the peripheral blood may not always reflect cells in tissue.[31] Future studies focusing on tryptophan degradation, gut microbiota changes, and Tc17/MAIT cells are warranted to clarify the direction of these associations and the potential impact for future therapeutic options.

ACKNOWLEDGMENT

The authors gratefully acknowledge the patients who made this study possible.

REFERENCES

1. 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.
2. Sousa AE, Carneiro J, Meier-Schellersheim M, et al. CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. *J Immunol.* 2002; 169:3400–3406.

3. Grossman Z, Meier-Schellersheim M, Sousa AE, et al. CD4+ T-cell depletion in HIV infection: are we closer to understanding the cause? *Nat Med.* 2002;8:319–323.
4. Hazenberg MD, Otto SA, van Benthem BH, et al. Persistent immune activation in HIV-1 infection is associated with progression to AIDS. *AIDS.* 2003;17:1881–1888.
5. Liu Z, Cumberland WG, Hultin LE, et al. 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.
6. Liu Z, Cumberland WG, Hultin LE, et al. 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.
7. Liu Z, Hultin LE, Cumberland WG, et al. Elevated relative fluorescence intensity of CD38 antigen expression on CD8+ T cells is a marker of poor prognosis in HIV infection: results of 6 years of follow-up. *Cytometry.* 1996;26:1–7.
8. Giorgi JV, Liu Z, Hultin LE, et al. Elevated levels of CD38+ CD8+ T cells in HIV infection add to the prognostic value of low CD4+ T cell levels: results of 6 years of follow-up. The Los Angeles Center, Multicenter AIDS Cohort Study. *J Acquir Immune Defic Syndr.* 1993; 6:904–912.
9. Brenchley JM, Schacker TW, Ruff LE, et al. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J Exp Med.* 2004;200:749–759.
10. Mehandru S, Poles MA, Tenner-Racz K, et al. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. *J Exp Med.* 2004;200:761–770.
11. Hunt PW. Th17, gut, and HIV: therapeutic implications. *Curr Opin HIV AIDS.* 2010;5:189–193.
12. 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.
13. Lim A, Tan D, Price P, et al. Proportions of circulating T cells with a regulatory cell phenotype increase with HIV-associated immune activation and remain high on antiretroviral therapy. *AIDS.* 2007;21: 1525–1534.
14. Kolte L, Gaardbo JC, Skogstrand K, et al. Increased levels of regulatory T cells (Tregs) in human immunodeficiency virus-infected patients after 5 years of highly active anti-retroviral therapy may be due to increased thymic production of naive Tregs. *Clin Exp Immunol.* 2009;155:44–52.
15. Kanwar B, Favre D, McCune JM. Th17 and regulatory T cells: implications for AIDS pathogenesis. *Curr Opin HIV AIDS.* 2010;5: 151–157.
16. Gaardbo JC, Nielsen SD, Vedel SJ, et al. Regulatory T cells in human immunodeficiency virus-infected patients are elevated and independent of immunological and virological status, as well as initiation of highly active anti-retroviral therapy. *Clin Exp Immunol.* 2008;154:80–86.
17. Sandler NG, Douek DC. Microbial translocation in HIV infection: causes, consequences and treatment opportunities. *Nat Rev Microbiol.* 2012;10:655–666.
18. Chevalier MF, Weiss L. The split personality of regulatory T cells in HIV infection. *Blood.* 2013;121:29–37.
19. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol.* 2004;4:762–774.
20. Favre D, Mold J, Hunt PW, et al. Tryptophan catabolism by indoleamine 2,3-dioxygenase 1 alters the balance of TH17 to regulatory T cells in HIV disease. *Sci Transl Med.* 2010;2:32ra36.
21. Kandanearatchi A, Brew BJ. The kynurenine pathway and quinolinic acid: pivotal roles in HIV associated neurocognitive disorders. *FEBS J.* 2012;279:1366–1374.
22. Perez-De La Cruz V, Carrillo-Mora P, Santamaria A. Quinolinic acid, an endogenous molecule combining excitotoxicity, oxidative stress and other toxic mechanisms. *Int J Tryptophan Res.* 2012;5:1–8.
23. Schwarz MJ, Guillemin GJ, Teipel SJ, et al. Increased 3-hydroxykynurenine serum concentrations differentiate Alzheimer's disease patients from controls. *Eur Arch Psychiatry Clin Neurosci.* 2013;263:345–352.
24. Platten M, Wick W, Van den Eynde BJ. Tryptophan catabolism in cancer: beyond IDO and tryptophan depletion. *Cancer Res.* 2012;72: 5435–5440.
25. Murray MF. Tryptophan depletion and HIV infection: a metabolic link to pathogenesis. *Lancet Infect Dis.* 2003;3:644–652.
26. Baban B, Chandler PR, Sharma MD, et al. IDO activates regulatory T cells and blocks their conversion into Th17-like T cells. *J Immunol.* 2009;183:2475–2483.
27. Guillot-Delost M, Le GS, Mesel-Lemoine M, et al. Human CD90 identifies Th17/Tc17 T cell subsets that are depleted in HIV-infected patients. *J Immunol.* 2012;188:981–991.
28. Gaardbo JC, Hartling HJ, Thorsteinsson K, et al. CD3+CD8+CD161high Tc17 cells are depleted in HIV-infection. *AIDS.* 2013;27:659–662.
29. Le BL, Martin E, Peguillet I, et al. Antimicrobial activity of mucosal-associated invariant T cells. *Nat Immunol.* 2010;11:701–708.
30. Cosgrove C, Ussher JE, Rauch A, et al. Early and nonreversible decrease of CD161++/MAIT cells in HIV infection. *Blood* 2013;121:951–961.
31. Gaardbo JC, Hartling HJ, Ronit A, et al. Regulatory T cells in HIV-infected immunological non-responders are increased in blood but depleted in lymphoid tissue and predict immunological reconstitution. *J Acquir Immune Defic Syndr.* 2014.
32. Gaardbo JC, Hartling HJ, Ronit A, et al. Different immunological phenotypes associated with preserved CD4+ T cell counts in HIV-infected controllers and viremic long term non-progressors. *PLoS One.* 2013;8:e63744.
33. Midttun O, Hustad S, Ueland PM. Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom.* 2009;23:1371–1379.
34. Byakwaga H, Boum Y, Huang Y, et al. The kynurenine pathway of tryptophan catabolism, CD4+ T-cell recovery, and mortality among HIV-infected Ugandans initiating antiretroviral therapy. *J Infect Dis.* 2014.
35. Hunt PW, Sinclair E, Rodriguez B, et al. Gut epithelial barrier dysfunction and innate immune activation predict mortality in treated HIV infection. *J Infect Dis.* 2014.
36. Annibali V, Ristori G, Angelini DF, et al. CD161(high)CD8+T cells bear pathogenetic potential in multiple sclerosis. *Brain.* 2011;134:542–554.
37. Billerbeck E, Kang YH, Walker L, et al. Analysis of CD161 expression on human CD8+ T cells defines a distinct functional subset with tissue-homing properties. *Proc Natl Acad Sci U S A.* 2010;107:3006–3011.
38. Dusseaux M, Martin E, Serriari N, et al. Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17-secreting T cells. *Blood.* 2011;117:1250–1259.
39. Walker LJ, Kang YH, Smith MO, et al. Human MAIT and CD8alphaalpha cells develop from a pool of type-17 precommitted CD8+ T cells. *Blood.* 2012;119:422–433.
40. Brenk M, Scheler M, Koch S, et al, von BD. Tryptophan deprivation induces inhibitory receptors ILT3 and ILT4 on dendritic cells favoring the induction of human CD4+CD25+ Foxp3+ T regulatory cells. *J Immunol.* 2009;183:145–154.
41. Jenabian MA, Patel M, Kema I, et al. Distinct tryptophan catabolism and Th17/Treg balance in HIV progressors and elite controllers. *PLoS One.* 2013;8:e78146.
42. Read MA, Cordle SR, Veach RA, et al. Cell-free pool of CD14 mediates activation of transcription factor NF-kappa B by lipopolysaccharide in human endothelial cells. *Proc Natl Acad Sci U S A.* 1993;90:9887–9891.
43. Vujkovic-Cvijin I, Dunham RM, Iwai S, et al. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. *Sci Transl Med.* 2013;5:193ra91.
44. Terness P, Bauer TM, Rose L, et al. Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: mediation of suppression by tryptophan metabolites. *J Exp Med.* 2002; 196:447–457.
45. Grohmann U, Fallarino F, Puccetti P. Tolerance, DCs and tryptophan: much ado about IDO. *Trends Immunol.* 2003;24:242–248.