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Interleukin 10 Responses Are Associated With Sustained CD4 T-Cell Counts in Treated HIV Infection

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Background. Inflammation persists in treated human immunodeficiency virus (HIV) infection and may contribute to an increased risk for non–AIDS-related pathologies. We investigated the correlation of cytokine responses with changes in CD4 T-cell levels and coinfection with hepatitis C virus (HCV) during highly active antiretroviral treatment (HAART).

Methods. A total of 383 participants in the Women's Interagency HIV Study (212 with HIV monoinfection, 56 with HCV monoinfection, and 115 with HIV/HCV coinfection) were studied. HIV-infected women had <1000 HIV RNA copies/mL, 99.7% had >200 CD4 T cells/μL; 98% were receiving HAART at baseline. Changes in CD4 T-cell count between baseline and 2–4 years later were calculated. Peripheral blood mononuclear cells (PBMCs) obtained at baseline were used to measure interleukin 1β (IL-1β), interleukin 6 (IL-6), interleukin 10 (IL-10), interleukin 12 (IL-12), and tumor necrosis factor α (TNF-α) responses to Toll-like receptor (TLR) 3 and TLR4 stimulation.

Results. Undetectable HIV RNA (<80 copies/mL) at baseline and secretion of IL-10 by PBMCs were positively associated with gains in CD4 T-cell counts at follow-up. Inflammatory cytokines (IL-1β, IL-6, IL-12, and TNF-α) were also produced in TLR-stimulated cultures, but only IL-10 was significantly associated with sustained increases in CD4 T-cell levels. This association was significant only in women with HIV monoinfection, indicating that HCV coinfection is an important factor limiting gains in CD4 T-cell counts, possibly by contributing to unbalanced persistent inflammation.

Conclusions. Secreted IL-10 from PBMCs may balance the inflammatory environment of HIV, resulting in CD4 T-cell stability.

Loss of CD4 T lymphocytes is central to the pathogenesis of HIV infection. Without intervention, helper T-cell function declines, followed by broad alterations in immune responses. HIV replication is efficiently controlled in most subjects by use of highly active antiretroviral therapy (HAART), which frequently results in undetectable HIV RNA and recovery of CD4 T-cell counts. However, a substantial proportion of treated subjects appear to have a higher risk for non–AIDS-defining illnesses and a life span that is estimated to be 10 years shorter, compared with HIV-negative populations [1–5]. Low CD4 T-cell counts during HAART are associated with increased risk of cancer, liver disease, and cardiovascular disease [6–8]. Persistent activation of CD4 and CD8 T cells, detected by expression of CD38 and HLA-DR, appears to be associated with limited recovery of CD4 T-cell counts [9]; current hypotheses suggest that disease progression may be more closely associated with immune activation levels than with HIV replication levels [10–12]. The

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trend for a higher risk of non–AIDS-related illnesses has also been observed among participants in the Women's Interagency HIV Study (WIHS), even in the presence of virologic control and initial recovery of CD4 T-cell counts [13–16].

Interleukin 10 (IL-10) is a type II cytokine initially characterized as an inhibitor of helper T-cell subtype 1 cytokines [17]. A major immunoregulatory effect of IL-10 is inhibition of antigen presentation, acting primarily on dendritic cells and monocytes [18, 19]. IL-10 may also decrease apoptosis of B and T lymphocytes [20–22]. As a consequence, several clinical studies have investigated treatment with recombinant IL-10 for subjects with inflammatory conditions such as psoriasis, Crohn disease, or rheumatoid arthritis [23].

Toll-like receptors (TLRs) are critical components of innate immunity and play a key role in pathogen defense [24]. TLR3, the receptor for double-stranded RNA, and TLR4, the receptor for bacterial lipopolysaccharide (LPS), have recognized roles in antiviral and antibacterial immunity [25, 26]. Bacterial products leaking from the intestinal mucosa [27] and residual HIV replication [28] may contribute to chronic inflammation in treated HIV infection. We investigated the ex vivo capacity of peripheral blood mononuclear cells (PBMCs) from HIV-infected women to respond to TLR3 and TLR4 ligands, representing innate responses to pathogens of intracellular or extracellular origin, respectively.

Dysregulated inflammatory cytokine responses in HIV-infected women have been reported in the WIHS [29, 30]. We observed that spontaneous release of inflammatory cytokines in vitro was demonstrable in >60% of HIV-infected women but that responses to TLR3 and TLR4 stimulation were reduced in women with HIV/hepatitis C virus (HCV) coinfection, compared with women with HIV monoinfection [29]. In contrast, IL-10 responses to TLR4 stimulation were enhanced in women with HIV monoinfection, compared with women with HIV/HCV coinfection or noninfected controls. High production of IL-10 in untreated HIV infection has been considered detrimental for virus control because of IL-10 inhibition of the antiviral activity of helper T-cell subtype 1 cytokines [31]. However, the implications of altered cytokine responses, including IL-10 production, in HAART recipients are incompletely understood. The aim of this analysis was to investigate the possible influence of cytokines produced by cells of the innate immune compartment on levels of CD4 T cells over time in treated HIV infection.

MATERIALS AND METHODS

Study Design and Subjects

This investigation was nested within the WIHS, a prospective multicenter study enrolling 3766 participants in 6 US cities (Brooklyn, NY; New York; Washington, DC; Chicago, IL; San Francisco, CA; and Los Angeles, CA) to investigate the natural and treated history of HIV infection in women [32, 33]. Participants are evaluated every 6 months and undergo laboratory testing, including measurement of CD4 T cell counts and plasma HIV RNA loads. PBMC samples are stored frozen and maintained in a comprehensive WIHS repository. HIV and HCV serostatus were determined at WIHS enrollment, 3–12 years before initiation of the current study.

Among HIV-infected women, the criteria for inclusion in this study were an HIV RNA load of <1000 HIV RNA copies/mL and a CD4 T-cell count of >200 cells/μL at 2 consecutive visits during the period from October 2003 through April 2004. The second of these 2 eligibility-defining visits was considered the baseline visit for this study. A group of HIV-uninfected but HCV-seropositive participants was also included for comparison because chronic HCV infection may contribute to chronic inflammation and because approximately 30% of HIV-infected women in the WIHS have HCV coinfection. A total of 383 participants were included (212 had HIV monoinfection, 115 had HIV/HCV coinfection, and 56 had HCV monoinfection). Almost all HIV-infected women (98%; 319 of 327) were receiving HAART at the baseline visit. Of the 8 participants not receiving HAART, 3 were receiving a combination of antiretroviral therapy without a protease inhibitor, and 5 were not taking antiretroviral therapy. Of the 171 women who were antibody positive for HCV, 164 (95.9%) were also tested for plasma HCV RNA. Among those tested, 129 (78.7%) had detectable HCV RNA. None of the HCV-positive women were receiving anti-HCV therapy.

Written consent was obtained from all subjects, and the institutional review boards from all sites approved the study.

PBMCs were used to evaluate in vitro cytokine responses to TLR3 and TLR4 stimulation. CD4 T-cell counts and HIV loads obtained at the same study visit were used as baseline values. For about 90% of participants (344), we identified a CD4 T-cell count 3–3.5 years after the baseline value was recorded and used this measurement to calculate changes in CD4 T-cell levels. A CD4 T-cell count measured 2–4 years after the baseline value was recorded was used for the remaining 10% of subjects (39).

Viral Loads

Plasma HIV-1 RNA levels were determined using the Nucleic Acid Sequence Based Amplification assay (NucliSens; bioMerieux), with a lower limit of detection of 80 copies/mL. HCV RNA load, determined at the enrollment visit, was detected by real-time polymerase chain reaction (PCR) assay using the Cobas Amplicor HCV Monitor 2.0 (Roche Diagnostics), with a dynamic range of 10–200 000 000 IU/mL.

Sample Processing and Storage

Blood, PBMC, and plasma samples were processed and stored according to standard WIHS protocols [32]. Briefly, PBMC and plasma specimens were obtained from peripheral blood

collected in cell-preparation tubes (Vacutainer; Becton Dickinson) containing sodium citrate as an anticoagulant. Separated plasma and PBMC specimens were stored in vapor-phase liquid nitrogen at the WIHS central repository (Sera Care Life Sciences, Frederick, MD).

Cytokine Secretion

PBMC culture conditions have been described elsewhere [29]. Briefly, parallel cultures (2×10^5 PBMCs/well) received TLR3 or TLR4 ligands (200 μg/mL polyinosinic:polycytidylic acid [pIC] for TLR3 and 20 ng/mL LPS for TLR4; Sigma). These concentrations of pIC and LPS and the duration of culture were established as optimal for cytokine secretion in preliminary experiments (data not shown). Supernatants from cultures with medium only were used to evaluate spontaneously secreted cytokines. Supernatants were recovered at 16 hours of culture and stored frozen until batch analysis using a Cytokine Bead Assay (Human Inflammation Kit; BD Biosciences) for simultaneous detection. The lower limits of detection were 12, 73, 3, 2.5, and 24 pg/mL, respectively, for interleukin 1β (IL-1β), interleukin 6 (IL-6), IL-10, interleukin 12 (IL-12), and tumor necrosis factor α (TNF-α). Cytokine values from individuals described elsewhere [29] who were seronegative for HIV and HCV were used as reference values for determining responder status. For IL-10 responses to TLR3 stimulation, a threshold of 70.8 pg/mL was used to classify responders (IL-10 level, >70.8 pg/mL) and low responders (IL-10 level, ≤70.8 pg/mL). The threshold for IL-10 response to TLR4 stimulation was 39.9 pg/mL. The threshold values were the lowest quartile responses detected in similar assays among HIV- and HCV-negative control PBMCs (Supplementary Table 1) [29].

Statistical Analysis

Cytokine response (ie, responder or low responder) status was determined at the baseline visit for each cytokine. Separate modeling was performed for TLR3-induced and TLR4-induced IL-10 responses because different biological factors are involved in each induction pathway. The Student *t* test was initially used to compare changes in CD4 T-cell counts (hereafter, "ΔCD4," defined as the difference between CD4 T-cell counts measured at baseline and counts measured 2–4 years later) among responders and low responders for each cytokine. Comparisons were further stratified on the basis of HCV and HIV infection status, with separate evaluation of HIV-monoinfected, HCV-monoinfected, and HIV/HCV-coinfected groups. Following these comparisons, subsequent analysis focused on the IL-10 response, using linear regression to compare ΔCD4 among IL-10 responders and low responders, with control for baseline CD4 T-cell counts. Other covariates, including race, baseline age, body mass index, smoking status, HIV status, and HCV status, were analyzed separately, with ΔCD4 as the dependent variable and with control for baseline CD4 T-cell count. HIV RNA level, HAART use, and nadir CD4 T-cell count were similarly analyzed in the HIV-positive subgroup. Factors found to be statistically significant were then included in multivariate analysis.

Multiple linear regression with the baseline IL-10 response as the primary independent variable included baseline CD4 T-cell level and HCV status as covariates. This multiple linear regression model was repeated for the HIV-positive subgroup, with further control for HIV RNA levels, HAART use, type of antiretroviral therapy used, and nadir CD4 T-cell count. An interaction term of IL-10 response and HCV status was evaluated in both the overall sample and the HIV-infected subgroup to assess whether the association between IL-10 response and ΔCD4 differed by the presence or absence of HCV coinfection. Stratified versions of the final multiple linear regression models further evaluated the association between IL-10 response and ΔCD4, stratified by HCV status, in the overall sample. In an alternative modeling approach, all CD4 T-cell measurements from the baseline visit to the final visit 2–4 years later were included as outcome variables in linear mixed models, specifying subject-specific random intercepts and slopes (regressing CD4 T-cell count over follow-up time). By use of the same independent variables as in the multivariate linear regression models, the association of baseline IL-10 response with the rate of change in CD4 T-cell count was tested. All analyses used SAS, version 9.1 (SAS Institute).

RESULTS

Characteristics of the Study Population

Characteristics of the 383 women included in the study are shown in Table 1. Race and body mass index were similar among study groups. HIV-monoinfected participants (n = 212) were younger than HCV-monoinfected participants (n = 56) and HIV/HCV-coinfected participants (n = 115) ($P < .0001$). The prevalence of current smoking was lower among HIV-monoinfected participants (27%), compared with HCV-monoinfected participants (79%) and HIV/HCV-coinfected participants (57%) ($P < .0001$). CD4 T-cell counts were similar in the groups with HIV monoinfection and HIV/HCV coinfection ($P = .18$). Among HIV-infected subjects, the frequency of detectable levels of HIV RNA (ie, >80 copies/mL), use of antiretroviral therapy, and time since initiation of HAART were similar between the HIV-monoinfected and HIV/HCV-coinfected groups. Evaluation of the TLR3-induced IL-10 response revealed that the mean ΔCD4 was significantly higher in responders, compared with low responders, overall ($P = .04$) and in HIV-monoinfected subjects ($P = .004$). Mean ΔCD4 did not differ by IL-10 response in the HCV-monoinfected group ($P = .85$) or the HIV/HCV-coinfected group ($P = .77$). Similar results for ΔCD4 were observed for the TLR4-induced IL-10 responses.

Table 1. Demographic Characteristics, Baseline Clinical Characteristics, and Changes in CD4 T-Cell Counts (ΔCD4) by Interleukin 10 (IL-10) Status Among Subjects in the Women's Interagency HIV Study

Characteristic	All Subjects (n = 383)	HIV-Monoinfected Subjects (n = 212)	HCV-Monoinfected Subjects (n = 56)	HIV/HCV-Coinfected Subjects (n = 115)	*P*
Age, y					
<40	125 (33)	102 (48)	10 (18)	13 (11)	<.0001
40–44	100 (26)	53 (25)	17 (30)	30 (26)	
45–49	83 (22)	34 (16)	14 (25)	35 (31)	
≥50	75 (19)	23 (11)	15 (27)	37 (32)	
Race					
African American	191 (50)	97 (46)	29 (52)	65 (56)	.54
Hispanic	112 (29)	69 (32)	17 (30)	26 (23)	
White	67 (18)	38 (18)	8 (14)	21 (18)	
Other	13 (3)	8 (4)	2 (4)	3 (3)	
BMI[a]					
≤24	116 (30)	65 (31)	12 (21)	39 (34)	.64
25–29	120 (31)	67 (32)	20 (36)	33 (29)	
≥30	141 (37)	76 (36)	24 (43)	41 (36)	
Missing	6 (2)	4 (1)	…	2 (1)	
Currently smoke					
Yes	167 (44)	58 (27)	44 (79)	65 (57)	<.0001
No	214 (56)	153 (72)	12 (21)	49 (43)	
Missing	2 (<1)	1 (<1)	…	1 (<1)	
CD4 T-cell count, cells/μL					
<350	66 (17)	36 (17)	0 (0)	30 (26)	<.0001
350–549	110 (29)	75 (35)	4 (7)	31 (27)	
550–749	98 (26)	61 (29)	6 (11)	31 (27)	
≥750	109 (28)	40 (19)	46 (82)	23 (20)	
HIV RNA load, copies/mL					
<80	274 (84)	181 (85)	…	93 (81)	.29
80–1000	53 (16)	31 (15)	…	22 (19)	
Therapy					
None	5 (1)	2 (1)	…	3 (3)	.26
Combination	3 (1)	1 (<1)	…	2 (2)	
HAART	319 (98)	209 (99)	…	110 (95)	
HAART duration, y					
<3	46 (14)	33 (16)	…	13 (12)	.73
3.0–4.9	62 (20)	39 (19)	…	23 (21)	
5.0–6.9	105 (33)	70 (33)	…	35 (32)	
≥7	106 (33)	67 (32)	…	39 (35)	
ΔCD4,[b] by IL-10 response[c]					
TLR3 induced					
≤70.8 pg/mL	26 (14)	23 (17)	44 (54)	21 (20)	NA
>70.8 pg/mL	81 (23)	111 (25)	25 (89)	34 (40)	
TLR4 induced					
≤39.9 pg/mL	22 (15)	21 (19)	31 (62)	25 (22)	NA
>39.9 pg/mL	70 (19)	93 (22)	49 (69)	21 (31)	

Data are no. (%) of subjects, unless otherwise indicated.

Abbreviations: BMI, body mass index; HAART, highly active antiretroviral therapy; HCV, hepatitis C virus; HIV, human immunodeficiency virus; NA, not applicable; TLR, Toll-like receptor.

[a] Defined as the weight in kilograms divided by the square of the height in meters.

[b] Defined as the change in CD4 T-cell count between baseline and follow-up, as described in Materials and Methods. Data are least squares mean no. of cells/μL (standard error of the mean). *P* values testing the differences in ΔCD4, by IL-10 response, among each of the 4 groups were 0.04, 0.004, 0.85, and 0.77, respectively, for TLR3, and 0.05, 0.01, 0.84, and 0.93, respectively, for TLR4.

[c] Defined as IL-10 secretion from peripheral blood mononuclear cells stimulated with the TLR ligands polyinosinic:polycytidylic acid (for TLR3) and lipopolysaccharide (for TLR4).

Table 2. Association Between Demographic and Clinical Characteristics and Changes in CD4 T-Cell Counts (ΔCD4), With Control for Baseline CD4 T-Cell Count, Among Subjects in the Women's Interagency HIV Study

	All Subjects (n = 383)			HIV-Positive Subjects (n = 327)		
Variable	No.	ΔCD4[a]	*P*	No.	ΔCD4[a]	*P*
Age, y						
<40	125	34 (21)	.28	115	35 (19)	.83
40–45	100	27 (23)		83	50 (22)	
45–50	83	85 (25)		69	55 (25)	
≥50	75	24 (27)		60	27 (26)	
Race						
African American	191	37 (17)	.81	162	38 (16)	.76
Hispanic	112	35 (22)		95	38 (21)	
White	67	51 (28)		59	50 (27)	
Other	13	96 (64)		11	104 (62)	
BMI[b]						
<25	116	25 (22)	.59	104	38 (20)	.19
25–29	120	56 (21)		100	70 (20)	
≥30	141	42 (20)		117	20 (19)	
Currently smoke						
No	214	50 (16)	.45	202	52 (14)	.21
Yes	167	31 (18)		123	22 (19)	
HIV status						
Negative	56	99 (35)	.08			
Positive	327	31 (13)				
HCV infection status						
Negative	212	44 (16)	.79	217	51 (14)	.25
Positive	171	37 (18)		110	24 (19)	
Baseline HAART use						
No	…	…		8	16 (72)	.72
Yes	…	…		319	42 (11)	
Changed HAART[c]						
No	…	…		94	74 (21)	.07
Yes	…	…		233	28 (13)	
HIV RNA						
Not detected	…	…		274	52 (12)	.03
Detected	…	…		53	−16 (28)	
Nadir CD4 T-cell count, cells/μL						
<250	…	…		164	60 (16)	.12
≥250	…	…		162	23 (16)	
IL-10 response,[d] pg/mL						
TLR3 induced						
≤70.8	277	26 (14)	.04	236	22 (13)	.005
>70.8	105	81 (22)		90	92 (21)	
TLR4 induced						
≤39.9	231	22 (15)	.05	200	22 (14)	.03
>39.9	152	70 (19)		127	72 (18)	

Abbreviations: BMI, body mass index; HAART, highly active antiretroviral therapy; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IL-10, interleukin 10; TLR, Toll-like receptor.

[a] Defined as the change in CD4 T-cell count between baseline and follow-up, as described in Materials and Methods. Data are least squares mean no. of cells/μL (standard error of the mean).

[b] Defined as the weight in kilograms divided by the square of the height in meters.

[c] Defined as any change in antiviral medications between baseline and follow-up .

[d] Defined as IL-10 secretion from peripheral blood mononuclear cells stimulated with the TLR ligands polyinosinic:polycytidylic acid (for TLR3) and lipopolysaccharide (for TLR4).

Factors Associated With ΔCD4: Analysis Adjusted for Baseline CD4 T-Cell Count

After control for baseline CD4 T-cell count only, ΔCD4 did not significantly differ by age, race, body mass index, current smoking, or HCV infection status either among all subjects or among HIV-infected subjects only (Table 2). Detectable HIV RNA at baseline among HIV-infected subjects was associated with a decline in CD4 T-cell count ($P = .03$). Nadir CD4 T-cell count, defined as the lowest pre-HAART CD4 T-cell count, was not significantly associated with ΔCD4. IL-10 responses to TLR3 and TLR4 stimulation were significantly associated with increases in CD4 T-cell counts overall and among HIV-infected subjects only. ΔCD4 was not significantly associated with in vitro IL-1β, IL-6, IL-12, or TNF-α responses in any group (Supplementary Table 1). CD4 T-cell levels at HAART initiation were not associated with IL-10 responses ($P = .37$ for TLR3-induced responses, and $P = .16$ for TLR4-induced responses).

Sustained CD4 T-Cell Recovery and IL-10 Responses: Multivariate Analysis

Factors found to be statistically significant in the baseline CD4 T-cell count–adjusted analysis of ΔCD4 among HIV-infected subjects were included in a multivariate analysis. This model, which also adjusted for baseline CD4 T-cell count and HCV infection status, confirmed that ΔCD4 was associated with IL-10 response to TLR3 stimulation ($P = .01$), lower baseline CD4 T-cell count ($P = .0004$), and undetectable HIV RNA at the baseline visit ($P = .04$, Table 3). Similar analyses of the data on TLR4 stimulation indicated significant associations between CD4 T-cell count increases and IL-10 response ($P = .05$), lower baseline CD4 T-cell count ($P = .0004$), and undetectable HIV RNA ($P = .04$). HCV infection status in this model was not significantly associated with ΔCD4 ($P = .53$ and .45 for TLR3 and TLR4, respectively).

Additional analysis with linear mixed models used all CD4 T-cell count measurements (range, 2–9 data points; median number of measurements/participant, 8) from baseline to the final visit to test for differences in the rate of change (rather than absolute change) in CD4 T-cell count by IL-10 response. After adjustment for HCV status and HIV RNA status as in Table 3, the mean rate of change in CD4 T-cell count was 21.9 cells/year among TLR3 IL-10 responders and 17.6 cells/year among TLR4 IL-10 responders, compared with mean rates of change of −0.3 and −0.8 CD4 T cells/year among TLR3 IL-10 low responders ($P = .001$) and TLR4 IL-10 low responders ($P = .003$) (Table 4).

Effect of Sporadic Detectable HIV RNA on ΔCD4

Data were stratified by HIV viremia during the study period, with one group having undetectable HIV RNA (load, <80 copies/mL) during the interval (2–4 years) between baseline and follow-up and another group having HIV RNA detected at any time during the interval. After adjustment for baseline CD4 T-cell count and HCV infection status, beta estimates for IL-10 response were higher and statistically significant in the subgroup with detectable HIV RNA ($P = .02$ for TLR3 and TLR4; Table 5). ΔCD4 did not differ by IL-10 response among subjects with sustained undetectable HIV RNA.

Table 3. Multivariate Analysis of Factors Associated With Changes in CD4 T-Cell Counts Among Subjects in the Women's Interagency HIV Study (WIHS)

Variable	Subjects, No.	Beta Estimate (SE)	*P*
TLR3			
IL-10 response,[a] pg/mL			
≤70.8	236	Reference	.01
>70.8	90	65 (25)	
CD4 T-cell count at baseline		−0.16 (0.04)	.0004
HIV RNA			
Not detected	274	Reference	.04
Detected	52	−62 (30)	
HCV status			
Negative	212	Reference	.53
Positive	114	−15 (23)	
TLR4			
IL-10 response,[a] pg/mL			
≤39.9	200	Reference	.05
>39.9	127	44 (23)	
CD4 T-cell count at baseline		−0.16 (0.04)	.0004
HIV RNA[b]			
Not detected	274	Reference	.04
Detected	53	−62 (30)	
HCV infection status[c]			
Negative	212	Reference	.45
Positive	115	−18 (23)	

The change in CD4 T-cell count is defined as the difference between baseline and follow-up measurements, as described in Materials and Methods.

Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus; IL-10, interleukin 10; TLR, Toll-like receptor.

[a] Defined as IL-10 secretion from peripheral blood mononuclear cells stimulated with the TLR ligands polyinosinic:polycytidylic acid (for TLR3) and lipopolysaccharide (for TLR4).

[b] The lower limit of detection of the HIV RNA assay was 80 copies/mL. A subject was considered to have detectable HIV RNA if the assay detected HIV RNA at least once between the interval when baseline and follow-up CD4 T-cell counts were recorded.

[c] Determined at enrollment into the WIHS.

Effect of HCV Coinfection on CD4 T-Cell Recovery

Analyses to further clarify the effect of HCV coinfection and IL-10 responses on ΔCD4 indicated that IL-10 responses to TLR3 and TLR4 were significantly associated with sustained CD4 T-cell gains among HCV-negative women ($P = .006$ and

Table 4. Rate of Change in CD4 T-Cell Count Among Subjects in the Women's Interagency HIV Study, by Toll-like Receptor (TLR)–Induced Interleukin 10 (IL-10) Response

TLR Type	Subjects, No.	Change, CD4 T Cells/Year, Mean (SE)		P^a
		Low Responders	Responders	
TLR3	326	−0.3 (4.0)	21.9 (6.2)	.001
TLR4	327	−0.8 (4.3)	17.6 (5.4)	.003

The IL-10 response is defined as IL-10 secretion from peripheral blood mononuclear cells stimulated with the TLR ligands polyinosinic:polycytidylic acid (for TLR3) and lipopolysaccharide (for TLR4).

[a] By linear mixed effects modeling, with adjustment for hepatitis C virus status and human immunodeficiency virus RNA status.

.02 for TLR3 and TLR4, respectively). However, ΔCD4 was not significantly different between IL-10 responders and IL-10 low responders among HIV-infected women who were also infected with HCV ($P = .78$ and .95 for TLR3 and TLR4, respectively; Table 6). Correlation analysis between ΔCD4 and IL-10 level as a continuous variable, with control for baseline CD4 T-cell count, confirmed an association with responses to TLR3 stimulation among all 383 subjects ($P = .03$) and among the 212 with HIV monoinfection ($P = .03$), whereas there was no significant association for HIV/HCV-coinfected women or participants with HCV monoinfection ($P = .82$ and .19, respectively). Similar analysis for TLR4 indicated only a trend for the overall group and the group with HIV mono-infection (for the overall group: $P = .10$ and $r = 0.08$; for the HIV mono-infected group: $P = .16$ and $r = 0.10$). ΔCD4 was not significantly different between IL-10 responders or low responders among HIV/HCV-coinfected women when stratified by detectable HCV RNA ($P = .47$ and .43 for TLR3 and TLR4, respectively).

Comparable IL-10 Responses to TLR3 and TLR4 Stimulation

Multivariate analyses showed similar findings for both TLR3 and TLR4 stimulation (Table 3). This was further confirmed by direct comparison of IL-10 responses to TLR3 and TLR4 ligands. Levels of secreted IL-10 in response to either receptor stimulation were comparable, and samples with robust responses to stimulation via one receptor also had good responses to the second receptor (Figure 1; $P < .0001$ and $r = 0.85$). Similar patterns were observed for all other cytokines investigated. Depletion experiments confirmed our earlier findings that removal of CD14+ cells abrogated IL-10 secretion [29], whereas depletion of CD19, CD3, or CD56 cells did not reduce IL-10 levels during TLR4 stimulation.

DISCUSSION

IL-10 responses to TLR3 and TLR4 stimulation were associated with increases in CD4 T-cell counts over 2–4 years. To our knowledge, this is the first report to identify an immunoregulatory cytokine associated in a protective manner with the fate of CD4 T cells in treated HIV infection. Current hypotheses maintain that inflammation is a major factor in the residual pathogenesis of HIV infection in subjects who are receiving effective HAART. In this study, PBMCs from subjects with IL-10 responses also secreted cytokines associated with inflammation, such as IL-1β, IL-6, and TNF-α. However, CD4 T-cell recovery was more frequently sustained in subjects with IL-10 responses, despite the simultaneous presence of inflammatory cytokines. Thus, the association of preserved IL-10 responses with sustained CD4 T-cell recovery more closely fits a model of controlled inflammation rather than a model in which inflammatory factors are absent or reduced. Consequently, subjects with inflammatory cytokine responses alone, in the absence of IL-10, may be more likely to experience a decline in CD4 T-cell count. Alterations in cytokine responses at the

Table 5 Association Between Interleukin 10 (IL-10) Response and Change in CD4 T-Cell Count Between Baseline and Follow-up Among Subjects in the Women's Interagency HIV Study, by Human Immunodeficiency Virus (HIV) RNA Detectability

Variable[a]	Subjects, No.	Beta Estimate (SE)	P
TLR3			
Undetectable HIV RNA,[b] by IL-10 response[c]	147		
≤70.8 pg/mL	106	Reference	.25
>70.8 pg/mL	41	39 (34)	
Detectable HIV RNA,[b] by IL-10 response[c]	179		
≤70.8 pg/mL	130	Reference	.02
>70.8 pg/mL	49	82 (34)	
TLR4			
Undetectable HIV RNA,[b] by IL-10 response[c]	147		
≤39.9 pg/mL	86	Reference	.90
>39.9 pg/mL	61	4 (32)	
Detectable HIV RNA,[b] by IL-10 response[c]	180		
≤39.9 pg/mL	114	Reference	.02
>39.9 pg/mL	66	70 (31)	

The change in CD4 T-cell count is defined as the difference between baseline and follow-up measurements, as described in Materials and Methods. The model adjusted for CD4 T-cell count at baseline and hepatitis C virus status.

Abbreviation: TLR, Toll-like receptor.

[a] The P value for interaction of IL-10 response level and HIV viremia status was 0.15 for TLR3 and 0.13 for TLR4.

[b] The lower limit of detection of the HIV RNA assay was 80 copies/mL. A subject was considered to have detectable HIV RNA if the assay detected HIV RNA at least once between the interval when baseline and follow-up CD4 T-cell counts were recorded.

[c] Defined as IL-10 secretion from peripheral blood mononuclear cells stimulated with the TLR ligands polyinosinic:polycytidylic acid (for TLR3) and lipopolysaccharide (for TLR4).

Table 6. Association Between Interleukin 10 (IL-10) Response and Change in CD4 T-Cell Count Between Baseline and Follow-up Among Subjects in the Women's Interagency HIV Study (WIHS), by Hepatitis C Virus (HCV) Infection Status

Variable[a]	Subjects, No.	Beta Estimate (SE)	*P*
TLR3			
HCV negative,[b] by IL-10 response[c]			
≤70.8 pg/mL	145	Reference	.006
>70.8 pg/mL	67	84 (30)	
HCV positive,[b] by IL-10 response[c]			
≤70.8 pg/mL	91	Reference	.78
>70.8 pg/mL	23	12 (45)	
TLR4			
HCV negative,[b] by IL-10 response[c]			
≤39.9 pg/mL	123	Reference	.02
>39.9 pg/mL	89	67 (29)	
HCV positive,[b] by IL-10 response[c]			
≤39.9 pg/mL	77	Reference	.95
>39.9 pg/mL	38	−2 (38)	

The model adjusted for CD4 T-cell count at baseline and HIV RNA status.

Abbreviations: HIV, human immunodeficiency virus; TLR, Toll-like receptor.

[a] The *P* value for interaction of IL-10 response level and HCV infection status was 0.21 for TLR3 and 0.17 for TLR4.

[b] Determined at enrollment into the WIHS.

[c] Defined as IL-10 secretion from peripheral blood mononuclear cells stimulated with the TLR ligands polyinosinic:polycytidylic acid (for TLR3) and lipopolysaccharide (for TLR4).

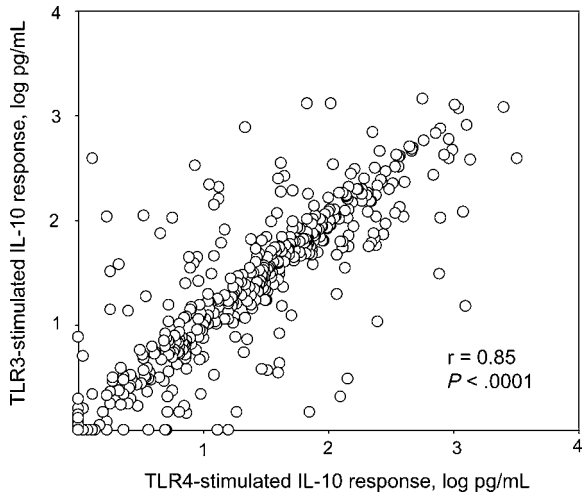

Figure 1. Interleukin 10 (IL-10) responses to Toll-like receptor (TLR) stimulation. Data are from culture supernatants that were tested for IL-10 secretion after peripheral blood mononuclear cells were stimulated for 16 hours with the TLR ligands polyinosinic:polycytidylic acid (for TLR3) and lipopolysaccharide (for TLR4).

level of innate immunity may also be associated with altered T cell memory responses, a possibility that is under investigation in our cohort. In this regard, T-cell activation is associated with lower gains in CD4 T-cell counts despite sustained HIV suppression, with persistent low-level viremia and with high levels of soluble markers of immune activation [9, 34, 35].

The association of IL-10 responses with sustained CD4 T-cell gains was not observed among HIV-positive women who were also coinfected with HCV, as analyzed by HCV antibody status or by HCV viremia. The impact of HCV coinfection on CD4 T-cell levels has been controversial, with some studies reporting compromised CD4 T-cell recovery in HAART-treated HIV/HCV-coinfected subjects [36–41] and with others finding no such association [34, 42–44]. Our results indicate that although there was a trend for lower gains in CD4 T-cell counts in HIV/HCV-coinfected women, these differences did not reach statistical significance. A possible explanation for this observation may be that HCV coinfection further burdens various components of the immune system that are already affected by HIV infection, resulting in more complex interactions. The HCV core, envelope, and NS3 proteins have all been described to affect interferon signaling, interferon-mediated responses, cytokine deregulation, and changes in apoptosis [45, 46]. It is also unclear from the current data whether chronic infections due to other pathogens, such as cytomegalovirus, herpes simplex virus 1, herpes simplex virus 2, and human papillomavirus, may contribute to the overall lymphocyte activation observed in our study. Importantly, while statistical significance for an association between ΔCD4 and IL-10 level was not reached in all groups, the trends related to IL-10 responses were similar for TLR3 and TLR4 stimulation in all groups, indicating functionality of both receptors. TLR3 and TLR4 represent innate responses to pathogen-associated molecular patterns of intracellular and extracellular origin, respectively, and thus resemble residual HIV replication and leakage of intestinal microbial products, which have been reported as 2 likely sources of persistent inflammation during chronic HIV infection.

Our results also indicate that intermittent increases of HIV RNA loads to low but detectable levels, also known as "blips," have a negative impact on CD4 T-cell increases, even in women with CD4 T-cell counts that have recovered to a clinically acceptable level (>200 cells/μL) after initiation of HAART. The effect of low but detectable levels of HIV RNA could specifically be addressed in this study because we included HAART-treated women with a baseline HIV RNA load of <1000 copies/mL. This observation is in agreement with findings from previous studies, in which blips were associated with lower CD4 T-cell counts [47–49]. Residual viremia may have an impact on lymphocyte activation, a hypothesis supported by studies showing a significant association

between detectable viremia (albeit with <50 HIV RNA copies/mL) with HLA expression on CD4 T cells [48]. Of note, intensification of antiretroviral therapy resulted in reduced expression of phenotypic markers of activation on CD4 and CD8 T cells [49]. Blips may reflect residual HIV replication in areas (eg, lymph nodes) that function as viral reservoirs [28] and where antiretroviral drugs may not reach the same therapeutic levels as in blood. Other factors, such as coinfections or variable adherence to HAART, can also impact residual replication. Interestingly, we found no association between ΔCD4 and nadir CD4 T-cell counts, suggesting that treatment successfully mediated CD4 T-cell recovery independently of nadir CD4 T-cell counts. The association of IL-10 responses with CD4 T-cell count increases in analyses stratified by HIV RNA load was stronger in subjects with detectable HIV RNA, compared with subjects with undetectable HIV RNA. This may indicate an effort of lymphocytes to dampen inflammation driven by blips, perhaps reflecting the function of cells with regulatory capacity.

The mechanisms by which IL-10 may favor sustained increases in CD4 T-cell counts are various. IL-10 has the capacity to limit cytokine secretion by modulating the activity of antigen-presenting cells [17–19]. Our data show simultaneous responses of both inflammatory cytokines and IL-10, but whether the IL-10 being produced was limiting expression of the inflammatory response or the activity of antigen-presenting cells is unclear and merits further investigation. Several cell types, including dendritic cells, monocytes/macrophages, B cells, T cells, and natural killer cells, can contribute to IL-10 production. In accordance with our preliminary observations [29], for TLR4, only CD14+ T cells appeared to be involved in IL-10 secretion in our 16-hour assay, as indicated from control experiments involving cell depletion. Since IL-10 may function as a paracrine or autocrine cytokine [19], we cannot exclude the possibility that cell types other than CD14+ T cells may synthesize IL-10 without secretion.

IL-10 has also been reported to inhibit apoptosis of both B and T lymphocytes [20–22], an effect mediated by upregulation of the bcl-2 protein [21, 50]. Whether IL-10 favors sustained CD4 T-cell counts by inhibiting lymphocyte apoptosis is an interesting possibility that is currently under investigation. Alternatively, it may be argued that sustained levels of CD4 T cells allow for the preserved IL-10 responses. However, our data indicate that in vitro failure of IL-10 stimulation by TLR3 or TLR4 ligands is detectable prior to the time that CD4 T-cell counts declined.

The pathogenesis of HIV infection includes development of severe immunodeficiency, and substances with broad immunosuppressive effects may exacerbate this condition, precluding their therapeutic application. The use of substances such as IL-10, which are immunomodulators and/or specifically enhance the activity of immunoregulatory factors, may provide new possibilities to control inflammation, leading to enhanced effectiveness of antiretroviral treatment over time.

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 copyedited. 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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