Correspondence

Characterization of Immune Failure by Monocyte Activation Phenotypes in HIV-Infected Patients Receiving Antiretroviral Therapy

To the Editor—Wilson et al [1] demonstrated that, in human immunodeficiency virus (HIV)–infected subjects, levels of inflammatory biomarkers known to be associated with increased clinical risk (interleukin 6, D-dimer, high sensitive reactive-C protein, soluble CD14, and soluble CD163) are more closely related to markers of monocyte activation and migration than to markers of T-cell activation. These data suggest that monocytes may play a prominent role in chronic and serious non-AIDS events, such as premature cardiovascular disease and cancer. Meanwhile, several large cohort studies have demonstrated that HIV-infected subjects who do not undergo full $CD4^+$ T-cell restoration during antiretroviral therapy (ART) (ie, those with immune failure [IF]) are at greater risk of developing these non-AIDS conditions [2–6].

Based on the findings of Wilson et al [1], we investigated monocyte activation phenotypes in a group of patients with IF, compared with patients who did obtain full $CD4^+$ T-cell recovery with therapy (immune success [IS]). We performed a cross-sectional study in 84 HIV-infected subjects with current $CD4^+$ T-cell counts <350/μL (IF; n = 39) or >500/μL (IS; n = 45) after ≥18 months of ART and with HIV RNA levels <50 copies/mL for ≥12 months. Peripheral monocyte/macrophage (M/M) phenotypes (based on CD14 and CD16 surface expression in classic $CD14^{++}CD16^-$, intermediate $CD14^{++}CD16^+$, and nonclassic $CD14^+CD16^{++}$ monocytes) as well as specific surface activation markers (eg, CD163 and CD11b) were evaluated by flow cytometry on fresh samples.

Patient demographic characteristics and immunological findings are provided in Table 1. The 2 groups were matched by both age and $CD4^+$ T-cell nadir. The IF and IS patient groups did not significantly differ by sex, duration of HIV infection, modality of transmission for HIV infection, Centers for Disease Control and Prevention stage, length of ART, or hepatitis B virus infection. The prevalence of hepatitis C virus infection was higher in patients with IF than in those with IS (38.5% vs 17.8%, respectively; $P < .05$). By definition, patients with IF displayed lower $CD4^+$ T-cell counts (median [interquartile range (IQR)] for IF vs IS, 268 [218–314] vs 663 [550–850] cells/μL; $P < .05$). A developmental relationship between circulating monocyte subsets (from classic by intermediate to nonclassic) might be postulated. During systemic infection and under proinflammatory stimuli, there is an increase first of the intermediate cells followed by the nonclassic $CD14^+CD16^{++}$ monocytes [7].

We found that, compared with patients with IS, those with IF showed lower levels of classic and higher levels of intermediate monocytes ($CD14^{++}CD16^-$, 82.5% of total $CD14^+$ [IQR, 77.0%–86.5%] for IF vs 84.5% [79.8%–88.3%] for IS [$P = .05$]; $CD14^{++}CD16^+$, 4.6% of total $CD14^+$ [3.2%–6.2%] vs 4.0% [2.5%–5.1%] [$P = .04$]). Proportions of nonclassic monocytes were also higher in patients with IF, although this difference did not reach statistical significance ($CD14^+CD16^{++}$, 6.7% of total $CD14^+$ [4.5%–9.6%] for IF vs 5.6% [3.3%–9.0%] for IS).

CD163 is a haptoglobin-hemoglobin scavenger receptor expressed by monocytes and macrophages, cleaved from the cell by proinflammatory stimuli and involved as soluble receptor (soluble CD163) in the regulation of inflammation [8]. Patients with IF displayed lower expression of CD163 than those with IS, for both total and classic monocytes ($CD14^+CD163^+$, 89.1% of total $CD14^+$ [IQR, 85.8%–91.9%] for IF vs 92.5% [88.3%–93.8%] for IS [$P = .02$]; $CD14^{++}CD16^-CD163^+$, 79.9% of $CD14^{++}CD16^-$ [74.0%–83.2%] vs 83.7% [78.9%–86.9%] [$P = .01$]). Although the values were higher in patients with IF, we found no significant differences between the 2 groups in the proportions of $CD14^{++}CD16^+CD163^+$ (4.3% of $CD14^{++}CD16^+$ [IQR, 3.0%–6.2%] for IF vs 3.9% [2.5%–5.1%] for IS; $P = .07$) or $CD14^+CD16^{++}CD163^+$ monocytes (1.9% of $CD14^+CD16^{++}$ [0.9%–2.6%] vs 1.2% [0.6%–2.5%]; $P = .35$).

CD11b (Mac-1), a member of the β2-integrin family, is involved in monocyte adhesion and endothelial transmigration as well as in macrophage activation. Constitutively present at low levels on the cell surface, its expression is increased after proinflammatory stimulation with cytokines (interleukin 1, tumor necrosis factor, and interferon γ) and lipopolysaccharide [9]. The frequency of $CD14^+CD11b^+$ did not differ substantially between the 2 groups (median, 98.1% of total $CD14^+$ [IQR, 96.8%–99.1%] for IF vs 97.8% [95.8%–98.8%] for IS; $P = .40$]). In contrast, patients with IF showed lower proportions of $CD14^{++}CD16^-CD11b^+$ (82.5% of $CD14^{++}CD16^-$ [IQR, 77.0%–86.5%] for IF vs 84.5% [79.8%–88.3%] for IS; $P < .05$) and higher proportions of $CD14^{++}CD16^+CD11b^+$ (4.6% of $CD14^{++}CD16^+$ [3.2%–6.2%] vs 4.0% [2.5%–5.1%]; $P < .05$) monocytes. Levels of nonclassic $CD14^+CD16^{++}$ monocytes expressing $CD11b^+$ did not differ significantly between the 2 groups (6.2% of $CD14^+CD16^{++}$ [IQR, 4.3%–8.9%] for IF vs 4.3% [2.9%–8.1%] for IS; $P = .08$). Moreover, we found no differences in the expression of HLA-DR,

Table 1. Epidemiological and Viroimmunological Data and Markers of Monocyte Activation[a]

Data and Markers	Immune Failure (n = 39)	Immune Success (n = 45)	*P* Value[b]
Epidemiological and viroimmunological data			
Age, y	51 (46–54.4)	48 (45–52)	NS
Male sex, No. (%)	33 (84.6)	38 (84.4)	NS
Previous AIDS diagnosis, No. (%)	31 (79.5)	35 (77.8)	NS
Duration of HIV infection, y	9 (4–20)	11 (6–21)	NS
$CD4^+$ T-cell nadir, cells/μL	49 (18–113)	87 (39–162)	NS
Duration of ART	6 (3–16)	9 (5–16)	NS
HCV positive, No. (%)	15 (38.5)	8 (17.8)	<.05
HBsAg positive, No. (%)	5 (12.8)	4 (8.9)	NS
Current $CD4^+$ T-cell count, cells/μL	268 (218–314)	663 (555–850)	<.05
Current $CD4^+$ T-cell proportion, %	17 (13–22)	29 (26–34)	<.05
Current CD4/CD8 ratio	0.36 (0.26–0.49)	0.76 (0.60–0.92)	<.05
Markers of monocyte activation			
$CD14^+$, %	6.24 (4.70–7.59)	6.12 (4.65–7.04)	NS
$CD14^{++}CD16^-$, % $CD14^+$	82.50 (77.00–86.55)	84.54 (79.76–88.33)	.05
$CD14^{++}CD16^+$, % $CD14^+$	4.61 (3.23–6.20)	4.03 (2.51–5.06)	.04
$CD14^+CD16^{++}$, % $CD14^+$	6.75 (4.49–9.60)	5.62 (3.31–9.02)	NS
$CD14^+CD163^+$, % $CD14^+$	89.08 (85.78–91.93)	92.46 (88.32–93.77)	.02
$CD14^{++}CD16^-CD163^+$, % $CD14^{++}CD16^-$	79.88 (74.03–83.22)	83.66 (78.99–86.86)	.01
$CD14^{++}CD16^+CD163^+$, % $CD14^{++}CD16^+$	4.26 (3.02–6.16)	3.91 (2.51–5.06)	NS
$CD14^+CD16^{++}CD163^+$, % $CD14^+CD16^{++}$	1.88 (0.86–2.61)	1.19 (0.62–2.46)	NS
$CD14^+CD11b^+$, % $CD14^+$	98.08 (96.84–99.06)	97.77 (95.77–98.85)	NS
$CD14^{++}CD16^-CD11b^+$, % $CD14^{++}CD16^-$	82.50 (76.98–86.55)	84.54 (79.76–88.33)	<.05
$CD14^{++}CD16^+CD11b^+$, % $CD14^{++}CD16^+$	4.61 (3.23–6.20)	4.03 (2.51–5.06)	<.05
$CD14^+CD16^{++}CD11b^+$, % $CD14^+CD16^{++}$	6.21 (4.30–8.95)	4.35 (2.86–8.06)	NS
$CD14^+HLADR^+$, % $CD14^+$	93.98 (89.38–96.21)	93.30 (91.37–96.23)	NS
$CD14^+CD38^+$, % $CD14^+$	98.37 (97.07–99.11)	98.44 (97.11–99.13)	NS
$CD14^+CD69^+$, % $CD14^+$	32.98 (20.27–50.12)	37.31 (22.26–58.28)	NS

Abbreviations: ART, antiretroviral therapy; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus; NS, not significant ($P > .05$).

[a] Unless identified as No. (%), data represent median (interquartile range) values.

[b] Statistical analyses were performed using Wilcoxon and χ^2 tests.

CD38, or CD69 on M/M between the 2 groups.

We found that suboptimal $CD4^+$ T-cell recovery during ART in patients with IF is associated with a shift of M/M phenotype from classic toward intermediate and nonclassic subsets, suggesting a role of these cells in the systemic immune activation typically observed in this group of patients.

The differential expression of CD163 and CD11b on monocytes observed in IF compared with IS could suggest a greater effort by the immune system to deal with this proinflammatory condition. Our findings provide further evidence that innate immune activation contributes to the pathogenesis of inflammation and poor $CD4^+$ T-cell restoration. Studies focusing on relationship between monocyte activation phenotypes and clinical end points (serious non-AIDS conditions) are indicated to evaluate the prognostic role of these cellular markers and potential strategies targeting persistent inflammation in the context of viral suppression.

Notes

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