

Elevated Spontaneous Interferon- γ Secretion in Human Immunodeficiency Virus-Infected Persons

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Immune activation in human immunodeficiency virus (HIV) is a well described phenomenon. We found that HIV patients have higher secretion of interferon (IFN)- γ compared with non-HIV subjects, as measured by the “nil” value in the QuantiFERON-TB Gold test, even when viral loads are low. This may reflect ongoing immune activation, even with optimal HIV management.

Keywords. HIV; immune activation; interferon- γ ; interferon- γ release assay; tuberculosis.

Persons with human immunodeficiency virus (HIV) experience immune activation that is chronic and beyond that expected by an intrinsic cellular response to the HIV virus alone [1]. Numerous mechanisms have been proposed, to include the following: (1) the CD4⁺ and CD8⁺ T-cell response to HIV, (2) activation of the innate immune system by translocation of gut bacteria after loss of T-cells in the gut mucosa and disruption of the mucosal barrier, (3) reactivation and replication of previously quiescent viruses (eg, Epstein-Barr virus and cytomegalovirus), and (4) direct immune cell activation through HIV gene products such as Nef or gp120 [1–4]. Interferons (IFNs) are master regulators of immune activation. Although IFN- α plays a key role in HIV host defense and is associated with immune activation in HIV-infected patients

[5, 6], IFN- γ is a central component of the innate and adaptive responses to viral infection, and reflects T-cell and natural killer cell activation, in addition to being implicated in chronic inflammatory and autoimmune states [7]. Interferon- γ release assays (IGRAs) are used to screen for *Mycobacterium tuberculosis* (MTB) infection in persons both with and without HIV [8]. These tests measure the amount of IFN- γ released from previously sensitized T-cells stimulated by MTB-specific antigens compared with the amount of IFN- γ released from nonstimulated blood (the “nil value”). We hypothesized that immune activation in HIV infection would be reflected by higher IFN- γ nil values in the QuantiFERON-TB Gold (QFT) IGRA compared with control subjects.

METHODS

We obtained QFT data on 1001 persons tested at HIV clinics (“HIV patients”) associated with the University of Washington (UW). We also obtained QFT data on 6241 persons tested at other UW sites (“controls”). These other sites included the following specialty clinics: transplant surgery (17%), arthritis (9%), occupational health (7%), and international (7%). Additional information was available for HIV patients including HIV viral load (VL), CD4 and CD8 counts, infection with hepatitis B or C, and comorbid inflammatory conditions (ie, cancer and autoimmune diseases). These data were obtained in collaboration with the Center for AIDS Research using specified laboratory tests and *International Classification of Diseases, Ninth Revision* codes. Student’s *t* test was used for continuous variables and the χ^2 test was used for categorical variables to evaluate for differences between groups. Multivariate linear regression was used to estimate the relationship between nil IFN- γ values and HIV, CD4 count, VL, and QFT result. Statistical analyses were performed using Stata (College Station, TX). The UW Institutional Review Board approved the study.

RESULTS

Of the 1001 HIV patients in this study, the majority (80%) had a single QFT measurement; 16% had 2 measurements and 3% had 3 or 4 QFT measurements. For ease of interpretation, the earliest QFT measurement was used in our analyses. In general, HIV patients were younger than control subjects at the time of first QFT testing (median age, 43 years [range, 17–74 years] vs 46 years [range, 1–95 years]; $P < .05$) and more likely to be male (85% vs 50%, $P < .05$). Results were similar when subjects were restricted to those at least 17 years old. Age, but not gender, was associated with geometric mean nil IFN- γ values in the combined dataset, although the magnitude of the effect was small (for every 10-year increase in age, the geometric mean nil value

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Table 1. Geometric Mean Nil IFN- γ Values in HIV Patients and Controls

Subject Group	Geometric Mean Nil IFN- γ Value	95% Confidence Interval
Controls (n = 6241)	0.094 IU/mL	0.092 IU/mL–0.097 IU/mL
HIV patients (n = 1001)	0.19 IU/mL	0.17 IU/mL–0.20 IU/mL
Stratified by QFT Result		
Controls, QFT negative (n = 5006; 80%)	0.088 IU/mL	0.086 IU/mL–0.091 IU/mL
Controls, QFT positive (n = 822; 13%)	0.16 IU/mL ^a	0.15 IU/mL–0.18 IU/mL
HIV patients, QFT negative (n = 915; 91%)	0.18 IU/mL ^a	0.16 IU/mL–0.19 IU/mL
HIV patients, QFT positive (n = 66; 7%)	0.49 IU/mL ^b	0.36 IU/mL–0.66 IU/mL
Stratified by HIV Viral Load		
Controls (n = 6241)	0.094 IU/mL	0.092 IU/mL–0.097 IU/mL
HIV patients, VL <200 (n = 45)	0.15 IU/mL ^c	0.11 IU/mL–0.21 IU/mL
HIV patients, VL \geq 200 (n = 280)	0.18 IU/mL ^c	0.16 IU/mL–0.20 IU/mL

Abbreviations: HIV, human immunodeficiency virus; IFN, interferon; QFT, QuantiFERON-TB Gold test; VL, viral load.

^a $P < .05$ (controls with negative QFT vs controls with positive QFT or HIV patients with negative QFT).

^b $P < .05$ (HIV patients with negative QFT vs HIV patients with positive QFT).

^c $P < .05$ (HIV patients with low or high viral loads vs controls).

decreased by 5%; $P < .05$). Control subjects were more likely than HIV patients to have either a positive QFT (13% vs 7%, $P < .05$) (Table 1) or an indeterminate QFT (7% vs 2%, $P < .05$). Due to the small number of subjects with indeterminate tests, they were not included in subsequent analyses. Positive QFT was associated with higher geometric mean nil IFN- γ values compared with those with negative QFT, in both HIV subjects and controls (Table 1).

Patients with HIV had higher geometric mean nil IFN- γ values compared with controls (0.19 IU/mL [95% confidence interval {CI}, 0.17–0.20 IU/mL] vs 0.094 IU/mL [95% CI, 0.092–0.097 IU/mL]; $P < .05$), even when VLs were less than 200 copies/mL (VL < 50 copies/mL: 0.14 IU/mL [95% CI: 0.055–0.34 IU/mL]; VL 50–199 copies/mL: 0.16 IU/mL [95% CI: 0.11–0.21 IU/mL]; VL 200–1000 copies/mL: 0.19 IU/mL [95% CI: 0.12–0.28 IU/mL]; VL > 1000 copies/mL: 0.18 IU/mL [95% CI: 0.16–0.20 IU/mL]; $P < .05$ when stratified by viral load \geq 200 copies/mL (Table 1). There was no relationship between nil IFN- γ values and CD4/CD8 ratio, CD4 percentage, cancer or autoimmune disease, hepatitis co-infection, serum globulin, or absolute number of monocytes or neutrophils among HIV-positive subjects. However, there was a positive relationship between the absolute lymphocyte count and nil IFN- γ values ($P < .05$). To further explore this, we evaluated the 325 HIV patients who had QFT, CD4, CD8, and VL measurement on the same date. Higher CD4 and CD8 counts were associated with increased geometric mean nil IFN- γ values (for every increase of 100 CD4 T cells, the geometric mean nil IFN- γ value increased by 6.9%; $P < .05$). Likewise, for every increase of 100 CD8 T cells, the geometric mean nil IFN- γ value increased by 2.4% ($P < .05$). All results were similar after adjustment for age, gender, and QFT result. Because T-cells are a major source of IFN- γ , this correlation validated differences at the low end of the assay standard curve as biologically

relevant. In multivariate models, positive QFT and greater CD4 count were independently associated with higher geometric mean nil IFN- γ values in HIV patients after adjustment for age, gender, and viral load ($P < .05$).

DISCUSSION

Our study demonstrates that HIV infection is associated with higher spontaneous secretion of IFN- γ . This was true even in HIV patients with negative QFT tests or low HIV VLs. This may reflect ongoing immune activation despite a low burden of active disease. In our study population, the majority of HIV-positive subjects had VLs above the limit of detection. Thus, we could not determine whether they would have QFT nil values similar to that of HIV-negative subjects if their HIV VLs were more optimally controlled.

The QFT nil values have previously been reported in several studies, including in the setting of US healthcare workers who converted from negative to positive IGRA [9], malaria infection in HIV-positive subjects [10], and in subjects with repeat IGRA tests [11]. Our report adds to this growing body of knowledge, and it is possible that the QFT nil value will have increased clinical and/or epidemiologic utility as it is further explored in a greater number of populations.

It is interesting to compare our results to a recent multi-national study by [12] that examined plasma concentrations of IFN- γ in a cohort of treatment-naïve HIV-positive subjects in low- and middle-income countries before and after ART therapy [12]. In that study, subjects with higher VL and lower CD4 count at baseline were more likely to have persistent elevation of IFN- γ after 24 weeks of ART therapy. However, those with persistently elevated IFN- γ at 24 weeks were somewhat more likely to have achieved virologic suppression compared to subjects with lower IFN- γ levels, and ongoing IFN- γ elevation was associated with a reduced risk of adverse clinical outcomes. Given

the cross-sectional nature of our study, we were unable to evaluate the temporal relationship between the nil QFT value and ART use or clinical outcomes. The two studies are also difficult to directly compare given differences in subject demographics and the lack of an HIV-negative control group in the study by Balagopal et al. However, their study suggests that persistently elevated IFN- γ levels in patients on ART may portend better long-term clinical outcomes in the setting studied by [12].

One strength of this study is the large number of HIV patients and control subjects, as well as available laboratory and clinical data on comorbid conditions, such as cancer or autoimmune disease, which might contribute to HIV-independent immune activation. Limitations include the lack of clinical or laboratory information about subject comorbidities in the control group. Because IFN- γ serum concentrations are elevated in some rheumatologic conditions, such as systemic lupus erythematosus (SLE) [13], a future study might compare QFT nil values of SLE patients to those of HIV patients with and without antiretroviral therapy. Likewise, one area of current investigation is the QFT nil value in subjects with macrophage activation syndrome/hemophagocytic lymphohistiocytosis, two conditions in which IFN- γ plays a known pathogenic role [14, 15].

CONCLUSIONS

Additional limitations include the absence of longitudinal data on HIV patients to determine the possible clinical relevance of increased serum IFN- γ and the inability to more fully evaluate the small number of subjects with indeterminate tests. However, despite these limitations, in light of the increasing evidence that chronic immune activation plays an important role in the accelerated development of age-related complications in HIV patients [4], our study adds weight to the argument that optimal control of HIV replication and virus-associated inflammation is required for the best clinical outcomes.

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