Anatomically Compartmentalized Human Immunodeficiency Virus Replication in HLA-DR$^+$ Cells and CD14$^+$ Macrophages at the Site of Pleural Tuberculosis Coinfection

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This study examined the impact of the host inflammatory microenvironment associated with localized tuberculosis (TB) on human immunodeficiency virus type 1 (HIV-1) replication within lymphocytes and macrophages in vivo. Paired plasma and pleural fluid samples from HIV-1–infected individuals with pleural TB ($n = 9$) were analyzed. Detection of host proteins incorporated into the HIV-1 envelope by immunomagnetic capture analysis provided insight into the phenotype of cells supporting HIV-1 replication. The results indicated that the 4.0-fold greater median HIV-1 load in pleural fluid, compared with median load in plasma ($P < .01$), was derived in part from viral replication within HLA-DR$^+$ cells, CD26$^+$ lymphocytes, and, importantly, CD14$^+$ macrophages. Greatly increased local concentrations of proinflammatory cytokines and immune activation markers in the pleural space correlated with the virologic findings. In summary, HIV-1 replication was increased at sites of *Mycobacterium tuberculosis* coinfection within activated cells, including lymphocytes and CD14$^+$ macrophages.

Opportunistic infections augment the generalized immune activation that drives viral replication and CD4$^+$ lymphocyte turnover in human immunodeficiency virus type 1 (HIV-1)–infected persons (reviewed in [1]). By promoting HIV-1 replication in this way, immune-activating stimuli may be associated with increases in systemic virus load [2–5], greater viral genotypic diversity [6, 7], accelerated disease progression [8, 9], and enhanced HIV-1 transmission risk [10].

Worldwide, tuberculosis (TB) is one of the most prevalent opportunistic infections in HIV-infected persons. Development of active TB is associated with immune activation [11–13] and increased HIV-1 load in the systemic circulation [2]. However, HIV-1 replication may be even more greatly enhanced at anatomical sites of active *Mycobacterium tuberculosis* (MTB) coinfection. In HIV-infected persons with tuberculous meningitis, virus load in cerebrospinal fluid is higher than that in blood [14], and, in those with pulmonary TB, HIV-1 load is greater in diseased lung segments than in segments that are not inflamed [15]. Indeed, induction of HIV-1 replication resulting from localized inflammatory stimuli may be markedly compartmentalized from replication occurring systemically [16].

Induction of HIV-1 replication by immune-activating stimuli in vivo also is detectable within body tissues and at the cellular level [17–19]. Genotypic analysis of HIV-1 within microdissected splenic white pulps revealed exquisite compartmentalization of HIV-1 quasi species, suggesting that highly localized enhancement of HIV-1 replication occurs in association with antigenic stimulation [19]. The relationship between activation of lymphocytes and HIV-1 replication in vitro is well characterized (reviewed in [20]), and levels of cell-free HIV-1 in vivo are thought to be maintained by continuous rounds of de novo infection in short-lived, activated lymphocytes [21]. However, reports are contradictory regarding the activating effects of MTB infection and bacterial lipopolysaccharide stimulation on HIV-1 entry and replication within cells of the monocytic lineage in vitro [22–31]. In vivo, cells of the monocytic lineage generally are thought to contribute little to the cell-free virus pool [32], although it is suggested that they are likely to become a more productive site of HIV-1 replication during opportunistic infections (reviewed in [33]). However, the results of 2 previous studies that aimed to determine whether macrophages serve as productive sources of viral rep-

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Informed written consent was obtained from all study participants before their inclusion in this study. The study protocol conforms with the human experimentation guidelines of the US Department of Health and Human Services and was approved by the Institutional Review Board at Case Western Reserve University, Cleveland, and the Ugandan National AIDS Research Subcommittee, Kampala, Uganda.

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lication in HIV-infected individuals with mycobacterial coinfection are contradictory [18, 34].

Since host cell-surface proteins are incorporated into the HIV-1 envelope during viral budding, HIV-1 acquires an envelope phenotype that reflects that of the host cell (reviewed in [35]). Analysis of cell-free HIV-1 in clinical specimens by immunomagnetic virus capture analysis, therefore, provides an extremely useful means of gaining insight into the phenotype of the cells supporting viral replication in situ [36]. In the present study, analysis of paired plasma and pleural fluid samples from HIV-1–infected individuals with pleural TB enabled us to determine the impact of the host inflammatory microenvironment on cellular compartments of HIV-1 replication at discrete sites of MTB coinfection. Correlation of the virologic data with measurements of concentrations of proinflammatory cytokines and immune activation markers provided important insights into the local impact of mycobacterial coinfection on HIV-1 replication.

Patients and Methods

Patients and clinical samples. Samples from HIV-1–infected patients with newly diagnosed pleural TB (TB/HIV) were selected from among those collected during a previous study conducted in Kampala, Uganda, by the Case Western Reserve–Makerere Research Collaboration [37]. Diagnoses of pleural TB were confirmed by culture of MTB or by finding caseating granulomas or acid-fast bacilli (or both) in pleural biopsy specimens. HIV-1 RNA concentrations in the plasma and pleural fluid samples had previously been measured (Z. Toossi, J. L. Johnson, R. A. Kanost, M. Wu, H. Luzze, P. Peters, A. Okwera, M. Jolobay, P. Mugyenyi, H. Aung, J. J. Ellner, and C. S. Hirsch, unpublished data) by use of the Amplicor HIV-1 Monitor Assay (version 1.5; Roche Diagnostic Systems). In the present study, paired plasma and pleural fluid samples from TB/HIV patients ($n = 9$) were selected if $\geqslant 6 \times 10^4$ HIV-1 particles were present in each sample. This is the minimum quantity of HIV-1 that permits immunomagnetic virus envelope phenotyping analysis, using $\geqslant 3$ different antibodies (2×10^4 virions per capture), as described below.

HIV-1 envelope phenotyping by immunomagnetic virus capture. Monoclonal antibodies bound to magnetic beads were used to target host proteins incorporated into the HIV-1 envelope and thereby selectively capture virus derived from specific cell pools, as described elsewhere [36]. Antibodies directed against CD14 and CD36 selectively capture virus derived from cells of the monocytic lineage, and monoclonal antibody directed against CD26 selectively captures virus derived from lymphocytes [36]. In addition, detection of HLA-DR incorporated in the HIV-1 envelope provides an index of the activation of the mononuclear cells (both lymphocytes and macrophages) supporting HIV-1 replication [13, 16, 38, 39]. Immunomagnetic virus capture using antibodies directed against CD44 (expressed by all mononuclear cells) and CD19 (expressed by B cells) provides the positive and negative controls, respectively [36].

As described elsewhere [36], HIV-1 in both plasma and pleural fluid samples was purified before virus capture to remove anti-HIV antibodies and other inhibitory proteins, such as acute-phase proteins and soluble forms of the targeted receptors. In brief, virus was pelleted by ultracentrifugation, was salt-treated by mixing with sodium chloride (final concentration, 0.5 *M*), and was purified by passage through Microspin S-400 HR sephacryl columns (Pharmacia Biotech). Sheep anti–mouse IgG magnetic beads (Dynal) were conjugated with monoclonal antibodies directed against the target host cell proteins (0.5 μg antibody/1.6×10^7 beads). Equal inputs of purified virus (2×10^4 virions) were incubated with each of the antibody-conjugated beads on a rotator at 4°C for 4 h in the presence of 5% normal human serum. Unbound virus was washed from the beads, virus specifically captured by the antibody-conjugated beads was lysed, and the HIV-1 RNA was quantified (Amplicor HIV-1 monitor test; Roche Diagnostic Systems).

Measurement of concentrations of immune markers. ELISAs were used to measure concentrations of proinflammatory cytokines and immune activation markers, including tumor necrosis factor (TNF)–α (Medgenix Biosource), interleukin-6, interferon (IFN)–γ, soluble (s) CD25, sCD14, β_2-microglobulin, and sTNF-receptor type I (sTNF-RI; R&D Systems). All assays were done according to the manufacturers' protocols.

Statistical analysis. Prism software (version 2.0; GraphPad Software) was used for the analysis. HIV-1 RNA concentrations and markers of immune activation approximated normal distributions. We used *t* tests to compare the concentrations of these variables in plasma and pleural fluid. Statistical significance was defined as $P \leqslant .05$.

Results

Patient characteristics. The HIV-1–infected study participants ($n = 9$) had a median age of 25.5 years (range, 22–35 years) and a median $CD4^+$ lymphocyte count of 164×10^6 cells/L (range, 34–374×10^6 cells/L). The median HIV-1 RNA concentration at the site of TB in pleural fluid (5.1 $\log_{10}$ RNA copies/mL; range, 3.0–5.8 $\log_{10}$ RNA copies/mL) was 4.0-fold greater than that in plasma (4.5 $\log_{10}$ RNA copies/mL; range, 2.4–5.3 $\log_{10}$ RNA copies/mL; $P < .002$).

Incorporation of HLA-DR into the HIV-1 envelope is increased in pleural fluid, compared with plasma. Immunomagnetic HIV-1 capture analysis, with antibody directed against CD44 (positive control), enabled a significant level ($\geqslant$3.0-fold over background) of HIV-1 to be captured from all samples; the mean levels of HIV-1 captured from plasma and pleural fluid samples by using this antibody were similar (figure 1). To address the hypothesis that MTB coinfection promotes HIV-1 replication in activated $HLA\text{-}DR^+$ mononuclear cells in situ, we compared the levels of cell-free HIV-1 bearing HLA-DR in plasma and pleural fluid samples. A significant level of HIV-1 was captured from all but one sample, using antibody directed against HLA-DR. However, in marked contrast to capture levels obtained when anti-CD44 antibody was used, the mean level of virus captured from pleural fluid when anti–HLA-DR antibody was used was significantly greater than that from plasma (figure 1). Calculation of the efficiency of virus capture confirmed that the mean proportion of the HIV-1 pool specifi-

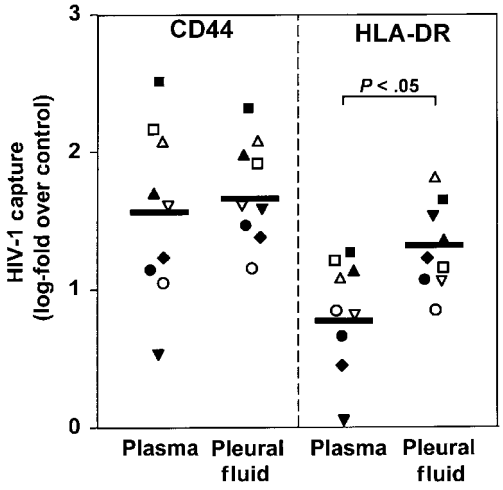

Figure 1. Increased capture of human immunodeficiency virus type 1 (HIV-1) from pleural fluid, compared with that from plasma, using anti–HLA-DR antibody. Equal amounts of HIV-1 (2×10^4 virions/capture) in paired plasma and pleural fluid samples obtained from patients with pleural tuberculosis and HIV-1 coinfection ($n = 9$) were analyzed by immunomagnetic capture. Monoclonal antibodies directed against CD44 (positive control), HLA-DR, and CD19 (negative control) were used. Different symbols for each patient indicate the level of HIV-1 captured by using antibodies to CD44 and HLA-DR, and bars show mean levels of virus capture. Although the mean levels of virus captured by using antibody directed against CD44 did not differ significantly between plasma and pleural fluid samples, the mean level of HIV-1 captured by antibody to HLA-DR was significantly greater from pleural fluid than from plasma ($P < .05$).

cally captured by anti–HLA-DR antibody ([anti–HLA-DR capture − anti-CD19 capture] × 100/virus input) was greater from pleural fluid (mean ± SE, 9.7% ± 2.1%) than from plasma (4.7% ± 0.7%; $P < .05$).

Lymphocyte- and macrophage-derived HIV-1. More extensive virus envelope analysis was done by immunomagnetic capture to gain insight into the cellular origin of HIV-1 present in the systemic circulation and in the pleural space. A greater quantity of virus was required for this analysis, and we were able to generate data from 8 of 9 pleural fluid samples, together with 5 of the paired plasma samples. Data from 2 representative patients are presented (figure 2). Using antibody directed against the lymphocyte-specific antigen CD26, a significant level of HIV-1 was captured from each of the plasma ($n = 5$) and pleural fluid ($n = 8$) samples tested. Thus, lymphocyte-derived virus was present in both anatomical compartments.

In contrast to lymphocyte-derived virus, there was strict anatomical compartmentalization of macrophage-derived HIV-1 captured by use of antibodies directed against CD36 and CD14 (figure 2). Macrophage-derived HIV-1 bearing CD14 in the envelope was detected exclusively within pleural fluid at the site of TB coinfection. In contrast, although HIV-1 bearing CD36 was detected in a proportion of both sample types, in individual patients this virus phenotype was present exclusively in either plasma or pleural fluid and never in both compartments (figure 2). The mean percentages of input HIV-1 captured by cell type–specific antibodies further illustrate the anatomical compartmentalization of macrophage-derived viruses bearing CD14 or CD36 in the virus envelope (table 1) and are similar in magnitude to those captured from analysis of plasma virus obtained from patients with pulmonary TB, as described elsewhere [36].

Concentrations of immune-activation markers in plasma and pleural fluid. Concentrations of proinflammatory cytokines and soluble immune markers in pleural fluid were elevated, compared with levels in plasma (figure 3), indicating intense local immune activation at the site of TB infection. IFN-γ [40]

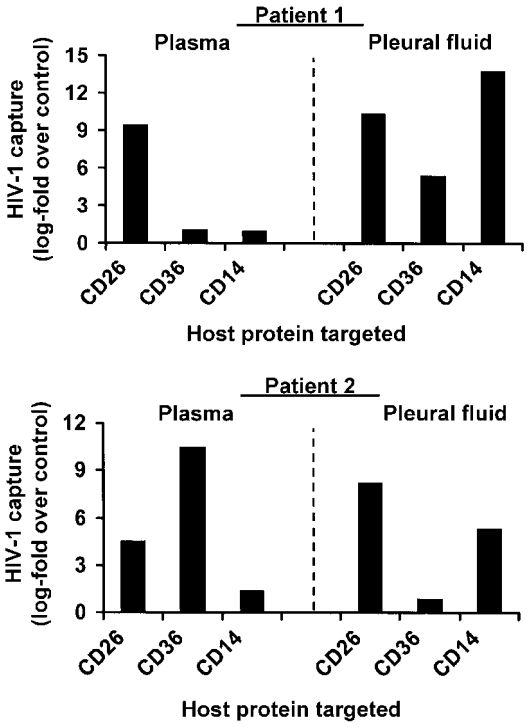

Figure 2. Detection of lymphocyte- and macrophage-derived human immunodeficiency virus type 1 (HIV-1) in plasma and pleural fluid samples from patients with pleural tuberculosis (TB). Immunomagnetic HIV-1 capture analysis was done, using monoclonal antibodies directed against CD26 (lymphocyte specific) and CD14 and CD36 (macrophage specific) host proteins incorporated into the HIV-1 envelope during budding. A significant level (≥3.0-fold over background) of HIV-1 was captured from all study samples by use of the positive control anti-CD44 antibody (figure 1). As illustrated by results for samples from 2 representative patients, lymphocyte-derived virus (bearing CD26) was detected in all pleural fluid ($n = 8$) and paired plasma samples ($n = 5$) tested. In marked contrast, HIV-1 bearing CD14 was present only at the site of *Mycobacterium tuberculosis* coinfection, being detected in 7 pleural fluid samples but in no plasma samples. Also, although HIV-1 bearing CD36 was detectable in 2 pleural fluid and 3 plasma samples, in each patient it was present exclusively in one compartment or the other. Thus, there was strict anatomical compartmentalization of macrophage-derived viruses, with $CD14^+$ being the dominant phenotype of macrophages supporting HIV-1 replication at the site of TB coinfection.

Table 1. Immunomagnetic capture of lymphocyte- and macrophage-derived human immunodeficiency virus type 1 (HIV-1) from plasma and pleural fluid samples from 5 patients coinfected with pleural tuberculosis and HIV-1.

Patient sample	Targeted host protein				
	CD44	CD26	CD36	CD14	CD19
Plasma	23.6 (8.6)	3.5 (1.4)	5.0 (2.5)[a]	1.0 (0.4)[a]	0.9 (0.5)
Pleural fluid	25.7 (6.0)	3.9 (1.6)	1.5 (0.9)[a]	6.9 (2.7)[a]	0.7 (0.3)

NOTE. Data are mean (±SE) percentage of input HIV-1 captured from paired samples from patients for whom data were available from analysis of both samples. Cell type–specific antibodies were used to target CD44 (positive control), CD26 (lymphocyte derived), CD36 and CD14 (macrophage derived), and CD19 (negative control) host antigens in the HIV-1 envelope. HIV-1 bearing CD14 was the predominant macrophage-derived virus type in pleural fluid, whereas HIV-1 bearing CD36 was the predominant macrophage-derived virus type in plasma.

[a] Differences in HIV-1 capture from plasma and from pleural fluid did not reach statistical significance ($P > .2$ for each).

and sCD25 [41] are derived predominantly from activated lymphocytes, and sCD14 is shed exclusively by cells of the monocytic lineage [42] in persons with HIV infection, TB, or dual infection [43]. Relative increases in concentrations of each of these markers in the pleural space, compared with plasma, indicate local activation of both lymphocyte and macrophage cell pools within the inflammatory microenvironment at the site of TB coinfection. Since elevated serum concentrations of sTNF-RI and β_2-microglobulin correlate independently with the presence of TB and HIV-1 infection, respectively [12], increased concentrations of these markers indicate a significant immunologic impact of each infection within the pleural space.

Discussion

The data in this study suggest that the inflammatory microenvironment associated with pleural TB has an important impact on HIV-1 replication in coinfected persons. The proinflammatory response in the pleural space was associated with compartmentalized increases in HIV-1 replication and detection of an increased proportion of the HIV-1 pool bearing HLA-DR within the virus envelope. In addition to lymphocytes, local $CD14^+$ macrophages made a significant contribution to the level of HIV-1 within the pleural space. Limited viral genotyping data also suggest that HIV-1 replication within activated $HLA\text{-}DR^+$ cells may lead to increased viral diversity (data not shown). Together, these data provide unique insight into the impact of opportunistic MTB infection on HIV-1 pathogenesis at active sites of inflammation.

Activated mononuclear cells expressing HLA-DR are the most productive source of HIV-1 replication in vivo [44]. HLA-DR is incorporated into the envelope of HIV-1 as it buds from either macrophages or lymphocytes [36, 38], and its presence in the virus envelope correlates with host cell activation [13, 16, 39]. We have shown previously that detection of HIV-1 bearing HLA-DR is greater in plasma of persons with pulmonary TB than in plasma of HIV-infected control persons with no opportunistic infection [13, 38]. The results of the present study extend this important observation, which indicates that MTB coinfection results in increased production of HIV-1 from activated $HLA\text{-}DR^+$ cells present within the inflammatory microenvironment at the site of disease. Since the presence of HLA-DR within the virus envelope enhances HIV-1 infectivity [45, 46], this phenomenon may represent an important additional mechanism whereby mycobacterial coinfections promote the local propagation of HIV-1, contributing to local increases in virus load. Moreover, interaction of virions bearing HLA-DR with T lymphocytes induces apoptosis [47], potentially increasing the frequency of apoptotic mononuclear cell death that occurs at sites of MTB and HIV-1 coinfection [37]. Despite the interesting observation that monocytes infected with high levels of MTB in vitro have reduced expression of HLA-DR [48, 49], HIV-1 replication at the site of the pleural TB in the present study is likely to have been induced in a wide pool of $HLA\text{-}DR^+$ cells, including activated lymphocytes and macrophages that were not infected by the bacterium.

Detection of both macrophage- and lymphocyte-derived HIV-1 in pleural fluid samples suggests that both mononuclear cell populations contributed to the increased virus load: median virus load was 4.0-fold greater in the pleural space than in plasma. Of importance, HIV-1 bearing the macrophage-specific antigen CD14 was detected in pleural fluid but in none of the plasma samples examined. The presence in the pleural space of virus derived from $CD14^+$ macrophages must have resulted from local replication at the site of TB infection and not from trafficking of virus from the systemic circulation. These data, therefore, indicate that mycobacterial coinfections have an important impact on HIV-1 replication within macrophages in vivo, which supports our earlier findings [36] and those of Orenstein et al. [18] and contradicts those of Van der Ende et al. [34]. Since macrophages represent long-lived reservoirs of HIV-1, increased infection within this cellular pool may contribute to sustained elevation of HIV-1 load [13], accelerated decline in immune function, and shortened survival [9] in HIV-infected persons treated for TB.

The striking compartmentalization of detectable HIV-1 with either CD14 or CD36 present in the virus envelope is likely to reflect differential expression of these antigens by cells of the monocytic lineage present in the 2 anatomic compartments. A key mycobacterial cell wall lipopolysaccharide, lipoarabinomannan, up-regulates CD14 expression by cells of the monocytic lineage [50], and engagement of this receptor by lipoarabinomannan induces TNF-α secretion [51] and HIV-1 transcription [28]. This provides a plausible mechanism by which the presence of MTB results in selective enhancement of HIV-1 replication within $CD14^+$ cells in the pleural space. Although HIV-1 bearing CD14 was not detected in the plasma of these HIV-infected individuals with pleural TB, it was detected earlier in those with pulmonary TB [36], possibly as a consequence of the marked lipoarabinomannan antigenemia that is present in persons with

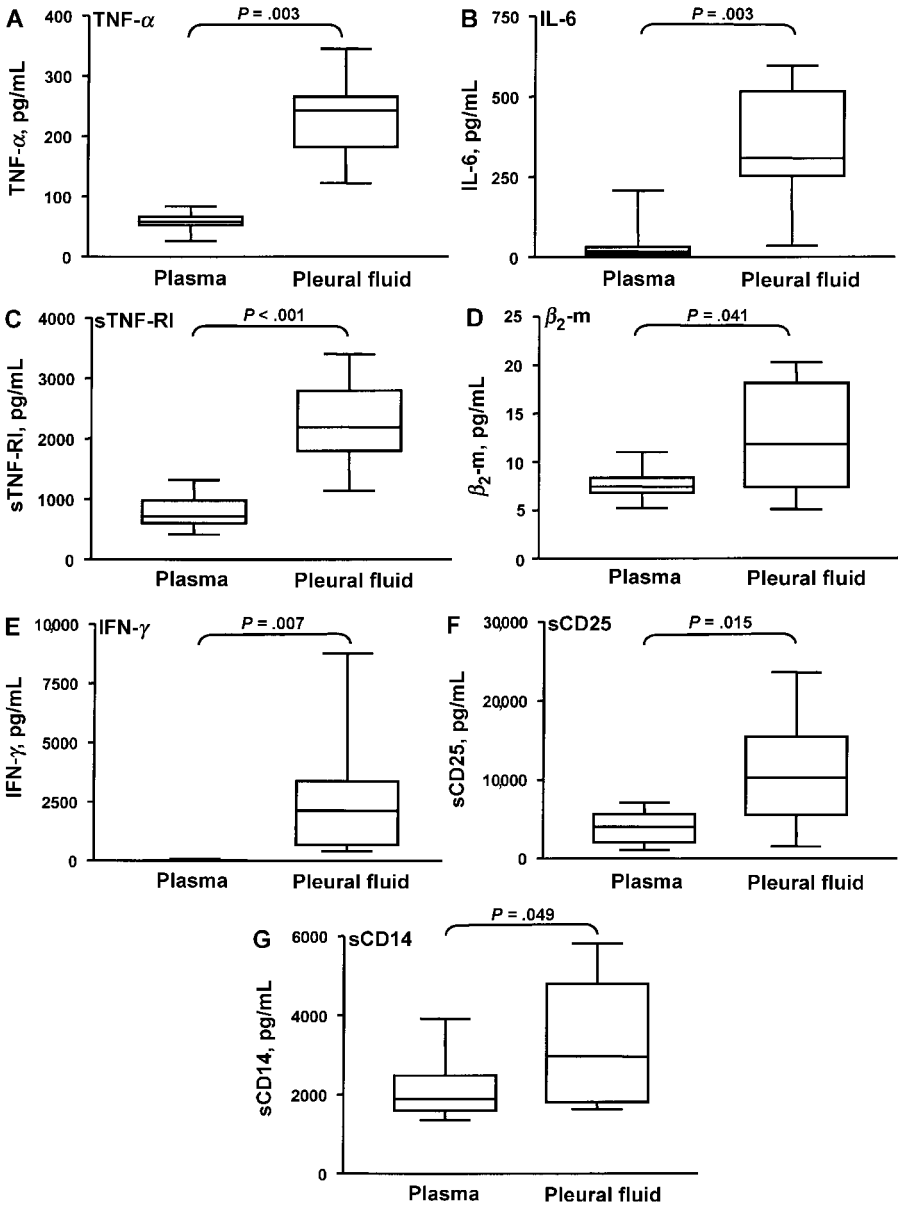

Figure 3. Compartmentalized immune activation within the pleural space in patients with pleural tuberculosis and human immunodeficiency virus type 1 coinfection ($n = 9$). Concentrations of tumor necrosis factor (TNF)–α *(A),* interleukin (IL)–6 *(B),* soluble (s) TNF–receptor type I (sTNF-RI; *C*), β_2-microglobulin (β_2-m; *D*), interferon (IFN)–γ *(E),* sCD25 *(F),* and sCD14 *(G)* were all significantly increased in pleural fluid, compared with plasma. Box and whisker plots indicate median values, 25th and 75th percentiles, and ranges of values.

pulmonary but not pleural TB [52]. In contrast to CD14, monocyte expression of CD36 is transiently up-regulated during the early phase of differentiation into macrophages, but it decreases with further cell maturation [53] and with cell exposure to bacterial lipopolysaccharide [54]. Thus, the finding of CD14 and not CD36 as the predominant macrophage-derived antigen in pleural fluid is likely to reflect induction of HIV-1 replication within local macrophages that were fully differentiated and were activated by exposure to mycobacterial products.

We suggested previously that the low efficiency of HIV-1 capture using antibodies to cell type–specific markers may be due, in part, to the inhibitory effect of plasma proteins and may reflect the inability to capture virus with an envelope antigen density below a critical threshold [36]. The efficiency of capture of in vitro–propagated stocks of macrophage- and lymphocyte-derived HIV-1, using antibodies to target CD36 and CD26, is also <25%, even in the absence of inhibitory plasma proteins [36]. Thus, it is likely that the proportion of macrophage-derived HIV-1 pres-

ent in plasma of persons with TB is substantially greater than the ~5% detected in this study. Limitations in capture efficiency restrict the immunomagnetic capture technique to making semiquantitative analysis of virus production from different cell pools, and the relative contributions of these pools cannot be compared directly, since the efficiencies of virus capture using various cell type–specific antibodies differ.

Increased concentrations of proinflammatory cytokines and soluble immune markers in the pleural space demonstrate the presence of marked immune activation at the site of MTB infection and correlate with the virologic findings. TNF-α and interleukin-6 synergistically increase HIV-1 replication at transcriptional and posttranscriptional levels [55, 56], and increased concentrations may have induced HIV-1 replication in the pleural space. Both IFN-γ and TNF-α up-regulate the expression of HLA-DR by mononuclear cells (reviewed in [57]), which supports the finding of increased incorporation of HLA-DR in the envelope of HIV-1 at the site of TB. Increased concentrations of cell type–specific soluble markers indicate activation of both lymphocytes and macrophages within the pleural space, corroborating the finding of virus derived from both cell populations in this compartment. Moreover, increased concentrations of sCD14 in pleural fluid also correlate with the frequent detection of CD14 in the envelope of macrophage-derived HIV-1 present within the pleural space.

In summary, the data in this study suggest that MTB coinfection in HIV-1–infected persons results in increased virus production from activated HLA-DR$^+$ mononuclear cells, including lymphocytes and CD14$^+$ macrophages present at the site of MTB infection. Increases in virus load and viral infection of long-lived macrophages, together with genotypic diversification, may contribute to acceleration of HIV-1 disease progression in persons with MTB coinfection.

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