Serum Immune Activation Markers Are Persistently Increased in Patients with HIV Infection after 6 Years of Antiretroviral Therapy despite Suppression of Viral Replication and Reconstitution of CD4+ T Cells

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The effect of long-term antiretroviral therapy on serum immune activation markers was assessed in a cohort of 63 patients before and after 6 years of boosted lopinavir–based antiretroviral therapy. High levels of most markers were associated with lower CD4+ T cell counts at baseline and at year 6, with the exception of soluble cytotoxic T lymphocyte antigen-4 (sCTLA-4); high levels of sCTLA-4 were associated with higher CD4+ T cell counts at year 6. Abnormalities of serum immune activation markers persisted after 6 years of ART but probably had different causes. Further investigation of the clinical usefulness of assaying immunoglobulin A, neopterin, and sCTLA-4 levels to assess the effectiveness of treatments for human immunodeficiency virus (HIV) disease are warranted.

Immune activation induced by human immunodeficiency virus (HIV) infection has several postulated causes, including activation of plasmacytoid dendritic cells (pDCs) resulting in production of interferon α (IFN-α) [1] and depletion of mucosal CD4+ T cells resulting in the translocation of bacterial products from the gut [2]. This results in the expression of activation markers, including CD38 and human leukocyte antigen (HLA)-DR, on the surface of T cells and increased levels of serum or plasma proteins, including neopterin, soluble tumor necrosis factor receptor (sTNFR) II and immunoglobulin (Ig) A [3, 4]. Assaying serum immune activation markers might provide additional information to the enumeration of activated T cells, because they are derived from different cell types (eg, neopterin from monocytes and macrophages and IgA from plasma cells) and reflect different aspects of HIV-induced immune dysfunction.

Treatment of HIV infection with combination antiretroviral therapy (ART) reduces immune activation, but immune activation may not return to normal. Ongoing immune activation is associated with poorer recovery of CD4+ T cells during early [5] and long-term ART [6] and might contribute to the pathogenesis of “non–AIDS-related HIV diseases,” such as atherosclerotic vascular disease and non–AIDS-related cancers [7]. Monitoring immune activation, in addition to monitoring CD4+ T cell counts, may therefore be clinically useful in treating patients who receive ART.

We have previously described patients with HIV infection who received an effective lopinavir and low-dose ritonavir–based ART regimen for 6 years, after which time, the proportions of activated (CD38+, HLA-DR+) CD4+ and CD8+ T cells were significantly lower than they were in untreated patients with HIV infection and comparable to what they were in HIV-negative subjects [8]. It is unknown what effect receiving ART for this length of time has on levels of serum immune activation markers or what relationship exists between serum immune activation markers and CD4+ T cell counts and T cell activation. We therefore assayed levels of 10 immune activation markers in serum samples obtained from these patients.

Patients and methods. Serum samples from 63 patients enrolled into Abbott study 720 [9], which were stored at baseline and years 3 and 6 of ART, were obtained for evaluation. All patients were ART naive when therapy was initiated with lopinavir and low-dose ritonavir with stavudine and lamivudine. Change of nucleoside analogues was allowed for drug toxicity. At year 6, all but one patient had a plasma HIV RNA level <50 copies/mL. Data on CD4+ T cell counts are presented in Table 1, and clinical data are presented elsewhere [9].

Enzyme-linked immunosorbent assays were used to evaluate serum levels of soluble cytotoxic T lymphocyte antigen-4 (sCTLA-4), IFN-inducible protein 10 (IP-10), monocyte che-
motactic protein 1 (MCP-1), and sTNFR-II (Biosource; In vitrogen Life Science); IFN-α and soluble TNF-related induced ligand (sTRAIL) (Diaclone; Tepnel Research Products and Services); and neopterin (Immuno-Biological Laboratories). Serum levels of IgG, IgM, and IgA were assayed by immunoturbidimetry (Abbott Architect 8200 Chemical Analyzer). Total and activated CD4+ and CD8+ T cells were enumerated by flow cytometry, as described elsewhere [8].

Serum levels of immune activation markers were summarized at baseline and years 3 and 6 using the median values and first and third quartiles. Changes from baseline to year 3 and 6 were assessed using a paired Student’s t test. Levels of immune activation markers were compared at each timepoint, using a 1-way analysis of variance, with levels of immune activation markers and CD4+ T cell count at year 6, using a stepwise selection process with a P value of ≤.05 required to enter and remain in the model.

Results. Data on serum immune activation markers in HIV-positive patients at baseline and years 3 and 6 of ART and comparison of values at these time points with those for non–HIV-infected subjects are presented in Table 1. At baseline, levels of all serum immune activation markers were increased in HIV-positive patients, compared with levels in non–HIV-infected subjects, with the exception of IgM and sCTLA-4. However, analyses of sCTLA-4 were complicated by the observation that 67% of patients had undetectable levels of sCTLA-4. At year 6, serum levels of IgG, IP-10, sTNFRII, neopterin, and sTRAIL were lower than baseline, but sTNFRII and sTRAIL levels remained higher in HIV-infected subjects than in non–HIV-infected subjects. Serum neopterin levels in HIV-infected patients could not be compared with levels in non–HIV-infected subjects, because the assay kits were not available.

Serum levels of IgA, IFN-α, and MCP-1 at year 6 were not statistically significantly lower than serum levels at baseline. Serum sCTLA-4 levels were <0.20 ng/mL in all 18 non–HIV-infected subjects, whereas serum levels were 0.20–2.5 ng/mL in 19 (33%) of 58 patients at baseline and in 17 (28%) of 61 patients at year 6, including 9 patients who had levels >0.2 ng/mL at both time points.

At baseline, levels of all serum immune activation markers except IgG, IgM, IFN-α, and TRAIL were correlated with CD4+ T cell counts (P<.05). All statistically significant correlations were negative, with the exception of sCTLA-4 level. At year 6, CD4+ T cell counts correlated negatively with baseline serum levels of IgA (R = −0.366; P = .004), MCP-1 (R = −0.293; P = .024), sTNFRII (R = −0.295; P = .023) and neopterin (R = −0.267; P = .043). In contrast, baseline serum levels of sCTLA-4 correlated positively with year 6 CD4+ T cell counts

Table 1. Clinical and Laboratory Values for Human Immunodeficiency Virus (HIV)–Negative Subjects and for HIV-Infected Case Patients at Baseline, Year 3, and Year 6

<table>
<thead>
<tr>
<th>Variable</th>
<th>HIV-negative control subjects (reference range)</th>
<th>HIV-infected case patients treated with lopinavir-ritonavir plus 2 NRTIs</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Year 3</td>
</tr>
<tr>
<td>CD4+ T cell count, cells/mm³</td>
<td>NA</td>
<td>237 (55–421)</td>
</tr>
<tr>
<td>HIV-1 RNA level, log₁₀ copies/mL</td>
<td>NA</td>
<td>5.1 (4.6–5.5)</td>
</tr>
<tr>
<td>sCTLA-4, ng/mL</td>
<td>0.116 (0.110–0.138)</td>
<td>0 (0–0.65)</td>
</tr>
<tr>
<td>IgA, mg/dL</td>
<td>178 (140–247)</td>
<td>249.5 (189–370)</td>
</tr>
<tr>
<td>IgG, mg/dL</td>
<td>992 (881–1129)</td>
<td>1413 (1169–1771)</td>
</tr>
<tr>
<td>Ip-10, pg/mL</td>
<td>122 (87–163)</td>
<td>106.5 (75.5–159)</td>
</tr>
<tr>
<td>IFN-α, pg/mL</td>
<td>0 (0–0.06)</td>
<td>5.9 (1.12–14.03)</td>
</tr>
<tr>
<td>IP-10, pg/mL</td>
<td>156 (93–502)</td>
<td>349 (188–480)</td>
</tr>
<tr>
<td>MCP-1, pg/mL</td>
<td>46.3 (25.1–53.6)</td>
<td>131.0 (81.2–180.0)</td>
</tr>
<tr>
<td>sTNFRII, ng/mL</td>
<td>3.50 (2.79–4.08)</td>
<td>11.0 (9.4–16.1)</td>
</tr>
<tr>
<td>Neopterin, nmo/L</td>
<td>NA</td>
<td>13.4 (9.9–24.1)</td>
</tr>
<tr>
<td>sTRAIL, pg/mL</td>
<td>307 (261–426)</td>
<td>1301 (963–1705)</td>
</tr>
</tbody>
</table>

NOTE. Data are mean value (interquartile range). IFN, interferon; Ig, immunoglobulin; IP-10, inducible protein 10; MCP-1, monocyte chemotactic protein 1; sCTLA-4, soluble cytotoxic T lymphocyte antigen 4; sTNF, soluble tumor necrosis factor receptor; sTRAIL, soluble tumor necrosis factor–related induced ligand.

* Statistically significant change from baseline value (P<.05).

b Statistically significantly different from reference value in HIV-negative subjects (P<.05).

c Data not available for HIV-negative control subjects because the enzyme-linked immunosorbent assay used for patient serum samples was not available at the time of testing.
(\(R = 0.355; P = .007\)), although as noted above, sCTLA-4 was undetectable in 67% of patients (Figure 1A). In multivariable analyses, adjusting for the impact of baseline CD4+ T cell count, the association between baseline serum levels of immune activation markers and CD4+ T cell counts at year 6 or change in CD4+ T cell count from baseline to year 6 were no longer statistically significant.

Plasma HIV RNA levels and CD4+ T cell counts correlated with serum immune activation markers to a similar degree at baseline (data not shown), with the exception of neopterin, which correlated more strongly with HIV RNA levels than did CD4+ T cell count (\(R = 0.55; P < .001\)).

At year 6, the serum IgA level correlated negatively with CD4+ T cell count (\(R = -0.338; P = .008\)) (Figure 1B), but it did not correlate with the proportion of activated CD4+ or CD8+ T cells. In contrast, the serum neopterin level correlated positively with the proportion of activated CD8+ (\(R = 0.362; P = .005\)) and CD4+ T cells (\(R = 0.289; P = .026\)) (Figure 1C) but not with CD4+ T cell counts. Furthermore, serum neopterin levels were higher in patients with activated CD4+ T cells >8% and/or activated CD8+ T cells >19% (ie, >2 standard deviations above mean levels) than in patients with activated CD4+ T cells <8% and activated CD8+ T cells <19% (8.1 nmol/L vs 17.0 nmol/L; \(P = .004\)). There was also a weak correlation between serum sTNFR-II levels and the proportion of activated CD4+ T cells (\(R = 0.263; P = .04\)).

**Discussion.** Increasing evidence suggests that treatments for HIV infection should suppress immune activation as well as increase CD4+ T cell counts. Assessment of immune activation by measuring proportions of activated CD4+ and/or CD8+ T cells may not provide information about all aspects of immune activation. Here, we have assayed levels of several serum immune activation markers in patients who received effective ART for 6 years, and we demonstrated that some markers might be clinically useful, as well as provide information about the characteristics of ongoing immune activation.

Serum IgA levels at baseline predicted CD4+ T cell counts at year 6 more strongly than any other serum immune activation marker and did not decrease after 6 years of ART, at which time they correlated negatively with CD4+ T cell counts. We have previously shown that serum IgA levels are negatively correlated with effector-memory CD4+ T cell responses in patients with increased CD4+ T cell counts who receive ART [10]. In contrast, we did not demonstrate a relationship between serum IgA levels and activated T cells after 6 years of ART. However, higher proportions of activated T cells were associated with higher serum neopterin levels. Because serum neopterin levels are considered to reflect activation of monocytes and macrophages [4], activation of T cells and monocytes and macrophages after 6 years of ART may have a similar cause but one that is different from the cause of high serum IgA levels.

Increased IgA production in patients with HIV infection during ART might reflect IgA antibody responses to lipopolysaccharides of gastrointestinal bacteria [11] as a consequence of failure to fully regenerate gut-associated lymphoid tissue (GALT) after long-term ART or increased production of cytokines that are switch factors for IgA-positive B cells, such as transforming growth factor \(\beta\) [12], which is produced by regulatory T cells that are increased in the blood of patients who are receiving ART [13]. An increased proportion of regulatory T cells and/ or failure to regenerate lymphoid tissue, represented by GALT,
might also impair the recovery of total and effector-memory CD4+ T cells.

We show here that higher serum levels of IgA, MCP-1, sTNFR-II, and neopterin at baseline predicted lower CD4+ T cell counts after 6 years of ART. However, in multivariable analyses, none of these serum immune activation markers was as effective as the baseline CD4+ T cell count in predicting low CD4+ T cell counts at year 6. Therefore, assaying serum immune activation markers provides no additional information to the CD4+ T cell count in predicting recovery of CD4+ T cells on ART.

Serum sCTLA-4 levels before and after 6 years of ART were higher in 28%–33% of HIV-infected patients than they were in non–HIV-infected subjects. Similar findings have been reported for patients with autoimmune thyroid disease [14], in whom increased serum sCTLA-4 levels appear to reflect production of a distinct transcript of the CTLA-4 gene. Interestingly, we found a positive correlation between serum levels of sCTLA-4 at baseline and CD4+ T cell counts at baseline and at year 6. CTLA-4 is an immunoregulatory receptor on B and T cells, and ligand engagement reduces T cell activation. sCTLA-4 may bind to ligands of CTLA-4, thereby preventing their engagement with cell-bound CTLA-4. This might suppress the inhibitory effect of CTLA-4 on T cell proliferation and result in increased CD4+ T cell proliferation. However, high serum sCTLA-4 levels might also have deleterious effects by increasing susceptibility to autoimmune disease, particularly autoimmune thyroid disease [14], which is one of several immune reconstitution disorders that may affect patients with HIV infection who receive ART [15].

The findings of our study add to the growing body of knowledge on the role of IFN-α in HIV-induced immune activation and suggest that PDC activation and/or dysfunction persist in patients who receive ART. As lymph node pDCs appear to be the major source of IFN-α in patients with HIV infection [1], assaying serum IFN-α levels might be the most convenient way of assessing this.

There are limitations to our study that should be considered. First, there may be serum immune activation markers not evaluated here that are more relevant than those that we investigated. However, we evaluated markers that appeared to be most informative, as determined by a review of previous publications. Second, the HIV-negative subjects were selected post hoc and were not matched with the HIV-positive patients by sex, age, or any other factor.

In summary, higher baseline serum levels of IgA, MCP-1, sTNFR-II, and neopterin were associated with lower CD4+ T cell counts in patients with untreated HIV infection and in patients who had received 6 years of effective ART. In contrast, higher serum levels of sCTLA-4 before ART initiation was associated with higher CD4+ T cell counts at year 6. After 6 years of effective ART, levels of most serum immune activation markers remained higher in HIV-infected patients than in non–HIV-infected subjects. At this time, IgA levels were negatively correlated with CD4+ T cell counts, and high serum neopterin levels were associated with T cell activation. We conclude that abnormalities of serum immune activation markers persist after long-term, effective ART, but they probably have different causes. Further investigation of the clinical usefulness of assaying serum levels of IgA, neopterin, and sCTLA-4 to assess the effectiveness of ART and other treatments for HIV disease are warranted.

References

10. French M, Keane N, McKinnon E, Phung S, Price P. Susceptibility to opportunistic infections in HIV-infected patients with increased CD4 T-cell counts on antiretroviral therapy may be predicted by markers of dysfunctional effector memory CD4 T cells and B cells. HIV Med 2007; 8:148–55.