Immunologic characteristics of healthy and HIV-1-infected Ethiopians

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CHAPTER 4

Plasma levels of viro-immunological markers in HIV-infected and -non-infected Ethiopians: correlation with cell surface activation markers
Plasma levels of viro-immunological markers in HIV-infected and non-infected Ethiopians: correlation with cell surface activation markers

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‡Department of Human Retro-Virology, Academic Medical Centre, University of Amsterdam, the Netherlands

Abstract

Cross-sectional studies were conducted to measure soluble viral and immunological markers in plasma in order to determine the prognostic value of these markers for HIV disease progression in Ethiopians and to see their association with cell surface markers in HIV-1-infected and -non-infected Ethiopians. Whole blood samples were collected from 52 HIV-1-negative Ethiopians; 32 HIV-1-positive Ethiopians with absolute CD4+ T-cell count >200/µl whole blood and no AIDS-defining conditions and 39 HIV+ Ethiopians with CD4+ T-cell count < 200/µl and/or AIDS-defining conditions. Plasma levels of β2-microglobulin (β2m), soluble CD27 (sCD27), soluble tumor necrosis factor-α receptor type II (sTNFR-II), IgG, IgA, IGE and IL-12 were elevated in HIV-1-infected individuals. The plasma levels of sTNFR-II, sCD27, β2m, IL-12 and IgG were inversely correlated with numbers of CD4+ T cells, the proportion of naive (CD45RA+CD27+) CD8+ T cells, and the proportion of CD8+ T-cells expressing CD28 (CD8+CD28+) and were positively correlated with the proportions of activated (HLA-DR+CD38+) CD4+ T-cells, as well as activated (HLA-DR+CD38+) CD8+ T-cells. A strong positive correlation was also observed when soluble immune markers were compared to each other. Multivariate regression analyses of soluble markers with numbers of CD4+ T cells showed that sCD27 is the best independent marker for CD4+ T-cell decline in the HIV-1-infected Ethiopians.

Our results indicate that measurement of soluble immune markers, which is relatively easy to perform, could be a good alternative to the quantification of T-cell subsets for monitoring HIV-1 disease progression in places where there is no facility for flowcytometric measurements.
Introduction

There is a wide variation in the course of disease among HIV-1-infected persons and thus considerable efforts have been put into the identification of factors that have a predictive value for the clinical stages of the disease, so-called surrogate markers. For example, the levels of HIV-1 replication (as expressed by viral loads) and the type of immune response during primary HIV-1 infection have a major impact on the ultimate course of HIV-1 disease (1-3). In addition, recent studies have indicated the association of levels of markers of immune activation and inflammatory cytokines with HIV-1 disease progression (4,5).

HIV-1 disease progression is reportedly faster and the time of survival after the onset of AIDS is shorter in African patients (6,7). Virologic and immune correlates of this altered disease course need to be identified by comparing HIV-1-infected and -non-infected Africans, as well as through prospective studies in cohorts of HIV-1-infected Africans in order to understand the course of HIV-1 disease and design therapeutic trials for this setting. However, there are still relatively little data available detailing surrogate markers and their use in monitoring HIV-1 disease progression in Africans.

We previously have compared several CD4+ and CD8+ T-cell subsets in HIV-1-infected and -non-infected Ethiopians to assess their potential use as predictors for HIV-1 disease progression (8). In summary, we observed a gradual increase of activated (CD38+HLA-DR) CD4+ and CD8+ T-cells, a decrease of CD8+ T-cells expressing CD28 and a decrease of effector (CD45RA+CD27) CD8+ T-cells when comparing HIV- subjects to HIV+ subjects and to the subjects with AIDS. Also, a decrease of naïve (CD45RA+CD27+) and an increase of memory (CD45RA+CD27+) CD8+ T-cells were observed in Ethiopians with AIDS.

Flowcytometry is not a widely available facility in Africa. On top of that, few laboratories in Africa have settings for manipulation of peripheral blood mononuclear cells (PBMC). Therefore, it was considered to be important to evaluate surrogate markers which can be measured using relatively cheap and easy to perform tests on plasma samples. In addition, surrogate markers reflect different biologic processes and their evaluation in African patients may contribute to the understanding of HIV disease pathogenesis.

In the present study we measured plasma viral load and several soluble immune markers and assessed their association with different stages of HIV-1 infection in Ethiopians. In addition, the relationship of soluble markers with previously established cell surface activation markers was assessed. Soluble CD27 (sCD27) is proposed to be the most suitable marker for monitoring HIV-1 disease progression in Ethiopians, when facilities for enumerating CD4+ T-cells are not present.
Plasma levels of viro-immunological markers

Materials and Methods

Subjects

The study population consisted of three groups of Ethiopians; 52 HIV-negative healthy individuals (designated "HIV-"), 32 HIV-positive individuals with CD4 counts >200/μl and no AIDS-defining conditions (designated "HIV") and 39 HIV patients with CD4 counts <200/μl and/or exhibiting AIDS-defining conditions based on the WHO staging system for HIV-1 infection and disease (9) (designated "AIDS"). The HIV and HIV' groups are factory workers participating in a prospective cohort study performed by the Ethio-Netherlands AIDS Research Project (ENARP) in Akaki, a village 15 km to the South-East of Addis Ababa, the capital of Ethiopia. This cohort study started in February 1997 after approval by both the National Ethical Committee and the EHNRI Ethical Committee. Cohort participants and their direct dependants are offered free medical care, according to the standards of the country. Subjects of the AIDS group are patients hospitalized in Addis Ababa, who were enrolled in order of their admittance to the hospital. HIV tests were performed by ENARP, upon request by the hospital clinicians, on coded blood samples. The blood samples were accompanied by a form detailing clinical information, gender and age only. HIV test results were returned to the hospital using the same codes. Patients in the hospital were pre- and post-test counselled and gave their informed consents, according to the hospital's policy. Samples were not included in the study when the quality of the sample was very poor, or when, according to the clinical information or whole blood CD4 T-cell counts, the criteria mentioned above were not met. No clinical history of the patients designated "AIDS" was available. Details of the subjects analysed in this study are as published (8).

Plasma isolation and viral load determination

Whole blood was collected in 10 ml EDTA Vacutainer tubes (Becton & Dickinson, USA) and plasma was isolated by centrifugation for 10 min at 300 g (1640 rpm) at room temperature, followed by storage at -80°C. HIV-1 RNA was isolated from the plasma as described (10).

Viral load was determined by quantifying the amount of HIV-1 RNA using a Nucleic Acid Sequence Based Amplification (NASBA) kit: NUCLISENS, Organon Teknika BV, Boxtel, The Netherlands). In our hands, the detection limit of this kit was 80 RNA molecules per ml of plasma. The NASBA methodology was previously shown to give quantitatively reliable results on HIV-1 subtype C plasma samples (11).

Measurement of soluble immunological markers

The following soluble immune markers were determined by commercial ELISA’s: sCD27 (Pelikine Compact human soluble CD27 ELISA kit, CLB, Amsterdam, The Netherlands), sTNFR-II (sTNF-R, 80 KDa ELISA, Bender
MedSystems, Vienna, Austria), IL-12 (Cytoscreen Immunoassay kit, BioSource International Inc., California, USA). $\beta_2$m, IgG, IgE and IgA were measured in plasma by immunonephelometric methods (DADE Behring, Marburg, Germany and CLB, Amsterdam, The Netherlands). All tests were performed according to the instructions of the kit manufacturers.

**Three color immunophenotyping of lymphocyte subsets**

Naïve and activated CD4$^+$ and CD8$^+$ T cells were measured using combinations of CD4/CD8$^+$ CD45RA and CD27 monoclonal antibodies (mAbs) or CD4/CD8$^+$ CD38 and HLA-DR mAbs, as described (8). Naïve and activated T cells were defined as CD45RA$^+$CD27$^+$ and CD38$^+$HLA-DR$^+$, respectively.

**Statistical methods**

Statistical analyses were performed using the STATA program (Stata Corporation, Texas, USA). The level of significance ($\alpha$), for the comparison of soluble markers between the three groups, was adjusted using Bonferroni correction ($\alpha=0.033$). The association between several soluble immune markers and CD4$^+$ T-cell count was determined using a multivariate regression analysis. Correlation coefficients were calculated by the Spearman's test.

**Results**

**Plasma levels of soluble immune markers and viral loads**

Fig. 1 summarizes the plasma levels of various soluble immune markers as measured on the three groups of Ethiopian subjects: HIV$^-$ (n=52), HIV$^+$ (n=32) and AIDS (n=39). In addition, log$_{10}$ viral load values are shown for HIV$^+$ and AIDS groups.

The levels of sCD27, $\beta_2$m and IL-12 were significantly and progressively increased when comparing HIV$^-$ to HIV$^+$, to AIDS groups: (Fig. 1a, 1b, 1c). Plasma sCD27 median values were 338 [278-392] U/ml (HIV$^-$), 406 [364-447] U/ml (HIV$^+$) and 761 [624-951] U/ml (AIDS). Plasma $\beta_2$m values were 1 [1-2] mg/L (HIV$^-$), 3 [2-4] mg/L (HIV$^+$) and 5 [3-7] mg/L (AIDS) and plasma IL-12 were 74 [57-88] pg/ml (HIV$^-$), 134 [82-257] pg/ml (HIV$^+$) and 197 [151-365] pg/ml (AIDS), respectively.

**Legend to Fig. 1** - Comparison of plasma levels of soluble immune markers between HIV$^-$, HIV$^+$ and AIDS groups. a) sCD27, b) $\beta_2$m, c) IL-12, d) IgE, e) IgG, f) IgA, g) sTNFR-II, h) log$_{10}$ viral loads. Data are expressed in 50 percentiles, with vertical bars, indicating 25 and 75 percentiles. The number of subjects tested is indicated, as well as the p-values of the various comparisons.
Plasma levels of viro-immunological markers 53

A

sCD27 U/ml

<table>
<thead>
<tr>
<th></th>
<th>HIV-</th>
<th>HIV+</th>
<th>AIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=42</td>
<td>25</td>
<td>51</td>
<td></td>
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B

B2-microglobulin mg/l

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<th>HIV-</th>
<th>HIV+</th>
<th>AIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=32</td>
<td>51</td>
<td></td>
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C

IL12 pg/ml

<table>
<thead>
<tr>
<th></th>
<th>HIV-</th>
<th>HIV+</th>
<th>AIDS</th>
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</thead>
<tbody>
<tr>
<td>n=25</td>
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<td></td>
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D

IgE IU/ml

<table>
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<th>HIV-</th>
<th>HIV+</th>
<th>AIDS</th>
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<tbody>
<tr>
<td>n=31</td>
<td></td>
<td></td>
<td></td>
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E

IgG g/l

<table>
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<tr>
<th></th>
<th>HIV-</th>
<th>HIV+</th>
<th>AIDS</th>
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<tbody>
<tr>
<td>n=30</td>
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<td></td>
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</table>

F

IgA g/l

<table>
<thead>
<tr>
<th></th>
<th>HIV-</th>
<th>HIV+</th>
<th>AIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=33</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

G

STNFR-II ng/ml

<table>
<thead>
<tr>
<th></th>
<th>HIV-</th>
<th>HIV+</th>
<th>AIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=39</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

H

Log HIV RNA copies/ml

<table>
<thead>
<tr>
<th></th>
<th>HIV+</th>
<th>AIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=38</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The other 4 soluble immune markers studied (IgE, IgG, IgA, TNF-R-II) were significantly increased in HIV\(^+\) versus HIV individuals, but did not show the above progressively increased values when comparing the 3 groups of Ethiopians (HIV, HIV\(^+\), AIDS). As shown in Fig. 1d, plasma IgE concentration was significantly increased in the HIV\(^+\) group (985 [98-7038] IU/ml), compared to HIV\(^+\) Ethiopians (481 [177-1126] IU/ml), but also to AIDS patients, (370 [174-1000]).

As shown in fig. 1e, the plasma levels of IgG were significantly increased in both the HIV\(^+\) (30 [21-36] mg/ml) and AIDS (25 [12-31] mg/ml) groups, compared to HIV Ethiopians (14 [12-17] mg/ml), with no significant difference between the two HIV–infected groups. Fig. 1f demonstrates that the increase in IgA concentration in the individuals with AIDS (3 [2-5] mg/ml) was significant as compared to the HIV\(^+\) group (2 [2-3] mg/ml), but not when compared to the HIV\(^+\) group (2 [2-4] mg/ml). Finally, as shown in fig. 1g, the levels of sTNFR-II were also significantly increased in the Ethiopians with AIDS (15 [10-21] ng/ml), compared to the HIV\(^+\) (5 [4-7] ng/ml) and HIV\(^+\) groups (5 [3-6] ng/ml). However, there was no significant difference in plasma sTNFR-II concentration between HIV\(^+\) and HIV\(^+\) Ethiopians.

As shown in fig. 1h, a significantly increased log\(_{10}\) viral RNA copy number was detected in the Ethiopians who developed AIDS (median 3.949 [1.903-5.122] log\(_{10}\) RNA molecules/ml plasma) compared to the HIV-1-infected asymptomatic Ethiopians (median 3.421 [1.903-4.384] log\(_{10}\) RNA molecules/ml plasma).

**Association of soluble markers with each other and with cell surface activation markers**

We investigated correlations of soluble markers with proportions of naïve and activated T-cell subsets. Table 1 indicates that the plasma levels of sTNFR-II, sCD27, \(\beta_2\)m, IL-12 and IgG were inversely correlated with the number of CD4\(^+\) T cells of the 3 groups, with proportions of naïve CD8\(^+\) T cells and with proportions of CD8\(^+\) cells expressing CD28. However, plasma levels of these soluble molecules were positively correlated with proportions of activated CD4\(^+\) and CD8\(^+\) T cells.

HIV-1 RNA load in plasma showed a tendency to negatively correlate with the number of CD4\(^+\) T cells. It showed also a moderate but positive correlation with proportions of activated CD4\(^+\) and CD8\(^+\) T-cells. We performed regression analyses to estimate the association of each of the soluble immune markers with the number of CD4\(^+\) T cells and plasma viral load in the HIV-1-infected individuals (HIV\(^+\) and AIDS) (Tables 2 and 3, respectively). Univariate analysis showed a significant association for sTNFR-II, sCD27, and \(\beta_2\)m with CD4\(^+\) T cells but plasma viral load was only significantly associated with sTNFR-II. Multivariate analysis revealed that sCD27 was the best independent marker for decrease in CD4\(^+\) T-cell count. However, plasma viral load was not associated with any of the soluble markers in multivariate analysis. Spearman correlation coefficients were also calculated to determine the association of the soluble markers with each other. Significant positive correlations were observed when plasma levels of sTNFR-II, \(\beta_2\)m, sCD27, IL-12 and IgG were correlated to each other (Table 4).
### Table 1: Spearman correlation coefficients (and p values) of comparisons between soluble markers, log$_{10}$ viral loads (HIV* and AIDS groups, n=71) and various T-cell subsets (HIV*, HIV" and AIDS groups, n=123).

<table>
<thead>
<tr>
<th>T-cell subsets</th>
<th>Viral load</th>
<th>sTNFR-II</th>
<th>sCD27</th>
<th>$\beta_2m$</th>
<th>IL-12</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of CD4* T cells</td>
<td>-0.25</td>
<td>-0.53</td>
<td>-0.64</td>
<td>-0.66</td>
<td>-0.59</td>
<td>-0.53</td>
</tr>
<tr>
<td>% CD8<em>CD28</em></td>
<td>-0.09</td>
<td>-0.40</td>
<td>-0.40</td>
<td>-0.44</td>
<td>-0.33</td>
<td>-0.31</td>
</tr>
<tr>
<td>% Activated CD4* T cells</td>
<td>0.34</td>
<td>0.48</td>
<td>0.69</td>
<td>0.73</td>
<td>0.69</td>
<td>0.66</td>
</tr>
<tr>
<td>% Activated CD8* T cells</td>
<td>0.31</td>
<td>0.46</td>
<td>0.69</td>
<td>0.73</td>
<td>0.70</td>
<td>0.63</td>
</tr>
<tr>
<td>% Naive CD4* T cells</td>
<td>-0.23</td>
<td>-0.08</td>
<td>-0.10</td>
<td>-0.06</td>
<td>-0.003</td>
<td>-0.09</td>
</tr>
<tr>
<td>% Naive CD8* T cells</td>
<td>-0.29</td>
<td>-0.39</td>
<td>-0.54</td>
<td>-0.44</td>
<td>-0.43</td>
<td>-0.33</td>
</tr>
</tbody>
</table>

### Table 2: Univariate and multivariate regression analyses of soluble immune markers and CD4* T-cell counts in -infected Ethiopians (HIV* and AIDS groups).

<table>
<thead>
<tr>
<th>Markers</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coeff.</td>
<td>95% CI</td>
</tr>
<tr>
<td>IgE</td>
<td>0.008</td>
<td>-0.003-0.02</td>
</tr>
<tr>
<td>IgG</td>
<td>0.17</td>
<td>-4.0-4.4</td>
</tr>
<tr>
<td>IgA</td>
<td>-17.0</td>
<td>-46.0-11.8</td>
</tr>
<tr>
<td>$\beta_2m$</td>
<td>-21.8</td>
<td>-40.2-3.4</td>
</tr>
<tr>
<td>sCD27</td>
<td>-0.40</td>
<td>-0.62-0.18</td>
</tr>
<tr>
<td>sTNFR-II</td>
<td>-8.7</td>
<td>-14.1-3.2</td>
</tr>
<tr>
<td>IL-12</td>
<td>-0.16</td>
<td>-0.53-0.20</td>
</tr>
</tbody>
</table>
Table 3: Univariate and multivariate regression analyses of soluble immune markers and log_{10} viral loads in HIV infected Ethiopians (HIV* and AIDS groups).

<table>
<thead>
<tr>
<th>Markers</th>
<th>Univariate</th>
<th></th>
<th></th>
<th>Multivariate</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coeff.</td>
<td>95% CI</td>
<td>P value</td>
<td>Coeff.</td>
<td>95% CI</td>
</tr>
<tr>
<td>IgE</td>
<td>0.00001</td>
<td>-0.00001-0.00007</td>
<td>NS</td>
<td>0.020</td>
<td>-0.023-0.065</td>
</tr>
<tr>
<td>IgG</td>
<td>0.0005</td>
<td>-0.024-0.025</td>
<td>NS</td>
<td>0.00003</td>
<td>-0.00004-0.0001</td>
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<tr>
<td>IgA</td>
<td>0.019</td>
<td>-0.143-0.182</td>
<td>NS</td>
<td>0.095</td>
<td>-0.176-0.368</td>
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<tr>
<td>β_{2m}</td>
<td>0.048</td>
<td>-0.056-0.154</td>
<td>NS</td>
<td>0.029</td>
<td>-0.237-0.178</td>
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<tr>
<td>SCD27</td>
<td>0.001</td>
<td>-0.0001-0.002</td>
<td>0.07</td>
<td>0.0002</td>
<td>-0.002-0.002</td>
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<tr>
<td>STNFR-II</td>
<td>0.033</td>
<td>0.002-0.065</td>
<td>0.03</td>
<td>0.031</td>
<td>-0.015-0.078</td>
</tr>
<tr>
<td>IL-12</td>
<td>0.002</td>
<td>0.00006-0.004</td>
<td>NS</td>
<td>0.001</td>
<td>-0.001-0.003</td>
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Table 4: Spearman correlation coefficients (and p values) of comparisons amongst soluble markers (HIV*, HIV* and AIDS groups, n=123) and log_{10} viral loads (HIV* and AIDS groups, n=71).

<table>
<thead>
<tr>
<th></th>
<th>sCD27</th>
<th>β_{2m}</th>
<th>IL-12</th>
<th>IgG</th>
<th>viral load</th>
</tr>
</thead>
<tbody>
<tr>
<td>sTNFR-II</td>
<td>0.79(&lt;0.001)</td>
<td>0.58(&lt;0.001)</td>
<td>0.55(&lt;0.001)</td>
<td>0.23(NS)</td>
<td>0.42(0.001)</td>
</tr>
<tr>
<td>sCD27</td>
<td>0.80(&lt;0.001)</td>
<td>0.67(&lt;0.001)</td>
<td>0.55(&lt;0.001)</td>
<td>0.31(0.02)</td>
<td>0.06(NS)</td>
</tr>
<tr>
<td>β_{2m}</td>
<td>0.72(&lt;0.001)</td>
<td>0.70(&lt;0.001)</td>
<td>0.63(&lt;0.001)</td>
<td>0.30(0.02)</td>
<td></td>
</tr>
<tr>
<td>IL-12</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Discussion

In Africa HIV-1 infection is spreading fast and has become one of the major causes of mortality (12,13). In Ethiopia the epidemic started relatively late, the first HIV-1-positive sera being detected in 1984 and the first AIDS patients reported in 1986 (14). However, since then the HIV-1/AIDS epidemic has spread to the entire country to reach a prevalence ranging 7-20% in the 15-50 year age groups in the urban areas by 1998 (15). The circulating subtype is C (16), which has been estimated to be the most prevalent (>48%) amongst HIV-1-infected individuals in the world (17).

Several studies have reported the accelerated progression to AIDS in HIV-1-infected Africans (6,7). Furthermore, recent reports more specifically pointed...
towards a possible more rapid HIV-1 disease progression in African women infected with subtype C viruses (18). Other reports did not detect a difference in HIV-1 disease progression when comparing HIV-1 subtype C-infected Ethiopians to HIV-1 subtype B-infected Caucasians in the same environment of the industrialized world (19).

In order to address the controversial issue of HIV-1 disease progression in Africans, surrogate markers need to be defined, which have prospective value in this context. Relatively little is known about surrogate markers in Africa (8,20-22). Altered disease progression might be partly explained by pre-existent immune activation, as a result of highly prevalent infectious- as well as parasitic diseases, including helminth infections (23-25) and also by absence of certain nutritional factors (26).

We previously have described several T-cell subset markers that could be of prognostic value for HIV-1 disease progression in Ethiopians. However, in many African laboratory settings expensive equipment and technical expertise required for flowcytometric analyses are not available. Therefore, there is a need for evaluation of markers, which can be measured using relatively cheap and easy methods. Several soluble laboratory markers, which are easily detectable in serum, have been shown to have a strong predictive value for HIV disease progression in other populations. Some of them were indicated to be as good as or sometimes even more accurate than numbers of CD4+ T cells as prognostic factors for the occurrence of AIDS (27-32). In this study, we assessed the plasma levels of soluble immune markers in HIV-1-infected and non-infected Ethiopians and we also investigated the association of these markers with proportions of various T-cell populations.

**Soluble markers progressively increasing with HIV status**

The levels of sCD27 in plasma showed a progressive increase in HIV-1-infected Ethiopians. Membrane-bound CD27 is expressed and released in a soluble CD27 form by activation of predominantly T cells (33). Although sCD27 is also present in biological fluids of healthy individuals, highly increased levels of sCD27 are detected in serum and urine of CMV patients, in cerebrospinal fluid of multiple sclerosis patients and also in serum and synovial fluid of patients suffering from rheumatoid arthritis (34,35). The strong association of sCD27 levels with the decline of the number of CD4+ T cells indicates that it could also be used as prognostic marker of HIV disease progression in addition to its previously described potential as disease progression marker in systemic lupus erythematosus (SLE) and B-cell leukemia (36,37).

The progressive increase of plasma β2m when comparing the Ethiopian HIV-, HIV+ and AIDS groups, is in accordance with literature, where β2m is described as a useful prognostic marker for HIV-1 infection in Africa (20,21). β2m functions as the light chain of class-I histocompatibility molecules (38). It is shed from the cell membrane during the continuous turnover of HLA molecules and serum and urine concentration are increased in various states of immune activation (39).

IL-12 has been described as a Th1 driver cytokine (40,41). In HIV-1
infection a shift from Th1 to Th2 cytokine production has been indicated and a role for IL-12 in disease progression has been postulated. Surprisingly, in our study, the level of IL-12 in plasma was significantly and progressively increased in HIV-1-infected Ethiopians compared to the HIV-1-negative group of Ethiopians. This is not in support of IL-12 mediating the postulated Th1 to Th2 shift (42).

Similar to what has been reported by Bentwich et al. (43), we observed highly elevated plasma IgE levels in HIV-negative Ethiopians, compared to HIV-1-negative Dutch subjects (data not shown). In HIV-1-infected Ethiopians the plasma concentration of IgE was found to be again higher than in HIV-1-negative subjects. This increase could be explained by the previously described association of elevated IgE levels in plasma with T-cell dysregulation and the high prevalence of opportunistic infections in patients with AIDS (44). Also, the nutritional status of people at the early stages of HIV-1 infection could play a role here (45). Although, we observed an increase of IgE in the HIV-1-infected Ethiopians, there was no correlation between numbers of CD4+ T cells and levels of IgE or IgA in plasma. Thus, the increase of IgE and IgA levels is most likely the result of polyclonal activation of B-cells during HIV-1 infection independent from numbers of CD4+ T cells in the blood. However, the level of IgG, which was found to be increased in HIV-1-infected Ethiopians, was inversely correlated with the number of CD4+ T cells and positively correlated with the other soluble immune markers.

The plasma concentration of sTNFR-II was significantly increased in the Ethiopians with AIDS compared to the HIV* and HIV- groups. The ligand for TNFRII, TNFα has been strongly implicated in the pathogenesis of HIV infection (46). The levels of TNFα in HIV-1-infected Ethiopians are much higher than in HIV-1-infected Caucasians and these levels further increase upon the development of AIDS (47). Over-expression of TNFα mRNA and spontaneous secretion of high levels of TN-α in culture were observed in PBMCs isolated from AIDS patients (5,48). However, TNF-α is rapidly cleared from the circulation and it is therefore frequently undetectable (49). In contrast to TNF-α, soluble TNFRs are very stable. Soluble TNFRs which are shed from neutrophils, activated T cells and monocytes as a result of higher level of TNF-α have been postulated to serve as binding proteins for transportation of TNF-α and for stabilization and prolongation of its bioactivity. A strong correlation between levels of sTNFR-II and TNF-α was shown (5). Although an elevated concentration of sTNFR-II is not specific for HIV-1 infection and is observed in many different disease processes, such as sepsis, malignancies and autoimmune disorders, the increase of sTNFR, especially of type II in serum, has been found to be a strong indicator of progression to AIDS in non-African populations [4,28]. The significant increase of sTNFR-II in the Ethiopians with AIDS may not only reflect immune activation but may also have pathophysiological significance related to the higher occurrence of opportunistic infections in this group.

The soluble immune markers we tested were significantly correlated with each other. Furthermore, we observed significant inverse correlations with the number of CD4+ T cells, with proportions of naïve CD8+ T cells and with proportions of CD8+ T cells expressing CD28. Also, a positive correlation was observed between soluble markers and proportions of CD4+ and CD8+ T cells
expressing CD38 and HLA-DR. Although some of the soluble markers are not specific for HIV infection, the observed associations between soluble markers and T-cell subsets may suggest that they all reflect the immunologic response to HIV infection. However, plasma viral load showed only a tendency to be correlated positively with activated and negatively with naïve CD4+ and CD8+ T cells. A moderate positive correlation was also observed between viral load and sTNFR-II as well as sCD27. The low correlation of viral load with soluble and cellular markers observed in this study may indicate that HIV viremia is not central to immune activation and HIV disease progression in Ethiopians.

Our results indicate that measurements of soluble immune markers in fresh or stored plasma, which are relatively easy to perform, could be good alternatives to quantification of T-cell subsets when flowcytometry is not available. Although sTNFR-II, sCD27 and β2m were negatively associated with the number of CD4+ T cells in univariate analysis, sCD27 showed the best independent association with CD4+ T-cell count in multivariate analysis. Thus, serial measurement of this marker in prospective studies may give useful prognostic information for HIV disease progression in Ethiopians.

In conclusion, the present study is meant to provide information as to which potential immune soluble markers (in addition to the T-cell subsets that we previously described) are useful in predicting HIV disease course in Ethiopians. The results of this study will help to select soluble markers that could be candidates for predicting HIV disease progression in an Ethiopian context, but which subsequently should be validated in a prospective studies.

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References

Plasma levels of viro-immunological markers 61


