Neopterin Estimation Compared with the Ratio of T-Cell Subpopulations in Persons Infected with Human Immunodeficiency Virus-1

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We measured neopterin, a biochemical indicator for the activation of cell-mediated immune reactions, in urines from 105 individuals at risk of infection with human immunodeficiency virus-1 (HIV-1), 83 of whom were seropositive for antibody to HIV-1. We compared absolute numbers of T-cell subsets (CD4+ helper/inducer T-cells, CD8+ suppressor/cytotoxic T-cells), and the ratio of CD4+ T-cells to CD8+ T-cells with the urinary neopterin concentrations. Concentrations of neopterin in urine were inversely correlated with absolute numbers of CD4+ T-cells and with CD4+/CD8+ ratios in anti-HIV-1 seropositive subjects but not in those seronegative. Various statistical comparisons of the data further demonstrated that neopterin concentrations showed larger differences between anti-HIV-1 seronegative and seropositive subjects than absolute numbers of CD4+ T-cells or CD4+/CD8+ ratios. These results seem to indicate that neopterin concentrations increase earlier in the course of HIV-1 infection, before effects on T-cell subpopulations are detectable, and may further support the suggestion that neopterin measurement could be of use for monitoring infected subjects or predicting the progression of disease.

Early prognostic information about the outcome of patients infected with human immunodeficiency virus-1 (HIV-1) is not easily obtained. HIV-1 selectively infects cells bearing CD4 molecules on their surface, so depletion of CD4+ helper/inducer T-lymphocytes is one of the most striking characteristics of the acquired immunodeficiency syndrome (AIDS). Therefore, quantification of specific T-cell subpopulations is widely used to monitor individuals infected with HIV-1. Changes of T-cell subsets are seen particularly in advanced HIV-1 infection and, with lower incidence, in asymptomatic seropositive individuals. Alterations in T-cell subpopulations were also reported in individuals of groups at risk: homosexuals, parenteral drug addicts, and hemophilia patients without established infection with HIV-1. Recent studies show that low numbers of T-helper cells and low ratios for T-cell subsets CD4+/CD8+ are significant predictors of disease progression to AIDS (1–3). However, quantification of T-cell subpopulations is expensive and laborious, and data on T-cell subpopulations reflect only circulating cells. Alternative tests of analytes that better represent the whole body’s immune status might be a substantial improvement.

Concentrations of neopterin, a biochemical marker for the activation of cell-mediated immunity, were consistently found to be increased in HIV-1-infected individuals (4). Here, we have compared neopterin concentrations with T-cell subpopulations (CD4+ and CD8+ T-cells), and the CD4+/CD8+ T-cell ratios in a selected population at increased risk for exposure to HIV-1. We studied the correlations between these variables in relation to the subjects’ anti-HIV-1 antibody status.

Materials and Methods

Patients: Included in the study were subjects who, in 1987, voluntarily attended the service institution of the Austrian AIDS-Hilfe in Vienna. They anonymously answered a questionnaire dealing with information about their sexual behavior and other activities associated with increased risk of being exposed to HIV-1, other infection episodes, and venereal diseases. In total, 105 adults (94 men, 11 women; mean age 30 y, range 19–52 y) were included: 63 homosexuals or bisexual males, 35 parenteral drug addicts, one hemophilia patient, and six individuals at high risk of heterosexual exposure to HIV-1. They all were screened for anti-HIV-1 antibodies (ELISA screening test; Abbott Laboratories, North Chicago, IL). Positive results were confirmed by Western blot and immunofluorescence tests. Eighty-three of these individuals were confirmed seropositive for antibodies to HIV-1. Of these, three were diagnosed as having AIDS, but did not present with acute opportunistic infections at the time they were being examined; seven had AIDS-related complex; and the remaining 73 subjects were free from additional AIDS-related symptoms, except for 44 with lymphadenopathy syndrome.

Thirty-three subjects were repeatedly tested, with at least three months between tests to minimize statistical bias from non-independence of observations. In all, we analyzed 278 samples of urine or blood for neopterin concentrations or T-cell subsets, respectively. Complete data for neopterin and T-cell subsets were obtained for 108 sample pairs from seropositive subjects and for 22 from seronegatives.

Specimens: We determined neopterin concentrations in samples of the first urine produced in the morning. The samples, protected from light by tin-foil covers, were stored at −30 °C until measurement. For quantification of T-cell subpopulations in heparinized whole blood, the samples were assayed without delay after collection.

Apparatus: We used a fully automated HPLC system (all from Varian, Palo Alto, CA): a Model LC 5500 liquid chromatograph, an air-activated auto-injection device (System 8055), a Fluorichrom fluorescence detector, and a UV 200 absorbance detector, all controlled by a Vista 402 data system. To quantify T-lymphocyte subpopulations after labeling of cells with monoclonal antibodies, we used a fluorescence-activated cell sorter (FACSCAN; Becton Dickinson, Mountain View, CA).

Assay methods: Neopterin was measured by HPLC as described earlier (5) but with slight updating modifications.
Briefly, the procedure was as follows. We diluted 100 μL of urine with 1 mL of Skrenson potassium phosphate buffer (15 mmol/L, pH 6.4) containing EDTA, 5.4 mmol/L. Diluted samples were injected by the automated sampling device onto 125 x 4 mm C18 reversed-phase columns (Lichrocart; Merck, Darmstadt, F.R.G.) through a 4-mm-long guard column packed with the same material and fitted in a column holder (Auto Fix II, Merck) at 25 °C. Compounds were eluted at a flow rate of 0.8 mL/min. Neopterin was monitored by its native fluorescence (535 nm excitation, 458 nm emission). To account for physiological variations in urine excretion, we related neopterin concentrations to urinary creatinine concentrations, which were quantified in the same chromatographic run by measuring the absorbance at 235 nm. With this procedure, analysis of one urine sample takes 8 min.

T-lymphocyte subpopulations, CD4+ helper/inducer T-cells, and CD8+ suppressor/cytotoxic T-cells were quantified according to standard techniques by fluorescence-activated cell sorter analyses with use of monoclonal antibodies Leu3 and Leu2, respectively (Becton Dickinson) after Ficol–Hypaque separation.

Statistical analyses: For statistical comparison of data between seronegative and seropositive individuals we used the Wilcoxon–Mann–Whitney U-test, because the data did not show a gaussian distribution. Spearman rank correlation coefficients were computed to test for correlations between variables. In addition, receiver-operating characteristic curves and Youden indices (Youden index = sensitivity + specificity – 1) were calculated and plotted for the whole range of all variables.

Results

There were significant positive correlations between the numbers of CD4+ T-cells and CD4+/CD8+ ratios, and between numbers of CD4+ and CD8+ T-cells. These correlations were not influenced very much by anti-HIV-1 antibody status (data not shown). In seropositive subjects but not in seronegative subjects, neopterin concentrations were inversely correlated with CD4+ T-cell numbers (Spearman rank correlation coefficient $r_s = -0.434$, $P < 0.0001$), with CD4+/CD8+ ratios ($r_s = -0.339, P < 0.0001$) and, albeit only weakly, with CD8+ T-cell numbers ($r_s = -0.214, P < 0.05$).

All four variables we investigated showed a significant difference between anti-HIV-1 seropositive and seronegative subjects (Table 1). The most striking differences between these groups were seen for neopterin concentrations, when U-values were compared.

Table 1. Statistical Comparison of Neopterin, CD4+ T-Cells, CD8+ T-Cells, and CD4+/CD8+ T-Cell Ratio in Anti-HIV-1-Antibody-Positive and -Negative Samples

<table>
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<tr>
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<th>Median (and 25 to 75 percentiles) in</th>
<th>Wilcoxon–Mann–Whitney U-test</th>
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<tr>
<td></td>
<td>HIV – (n = 22)</td>
<td>HIV + (n = 108)</td>
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<tr>
<td>Neopterin, μmol per m mole creatinine</td>
<td>153 (132–167)</td>
<td>303 (239–459)</td>
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<tr>
<td>CD4+/CD8+ T-cells ratio</td>
<td>1.71 (1.35–2.32)</td>
<td>0.785 (0.509–1.16)</td>
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<tr>
<td>CD4+ T-cells per µL</td>
<td>989 (770–1721)</td>
<td>685 (392–1145)</td>
</tr>
<tr>
<td>CD8+ T-cells per µL</td>
<td>594 (471–773)</td>
<td>810 (586–1276)</td>
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Figure 1 shows receiver-operating characteristic curves and Figure 2 shows Youden indices again for urinary neopterin concentrations (maximal Youden index = 0.77; optimal discriminator: 202 μmol of neopterin per mole of creatinine, which approximates the upper limits of normal in healthy heterosexuals (5)), and for CD4+/CD8+ T-cell ratios (maximal Youden index = 0.69; a broader range of similarly well discriminating values between ratios from 0.95 to 1.15). For absolute CD4+ and CD8+ T-cell counts, we found very broad plateaus with maximal Youden indices of 0.39 and 0.35, respectively.

Discussion

Neopterin is a sensitive marker for activated cell-mediated immunity. It is produced by human macrophages specifically on stimulation with gamma interferon (4). From its source, one would infer that neopterin is not specific for one single disease. Rather, increased concentrations are indeed seen in a panel of diseases and conditions that are associated with the activation of cell-mediated immunity (4).

High frequencies of increased neopterin concentrations in body fluids of HIV-1-infected individuals have been reported (4). In the present study, we have compared urinary neopterin concentrations with numbers of T-cell subpopulations and with the CD4+/CD8+ ratios in individuals apparently at risk of exposure to HIV-1. Anti-HIV-1 testing had been performed on these volunteers.

We found significant inverse correlations between neopterin concentrations and absolute numbers of CD4+ T-cells and CD4+/CD8+ ratios. These correlations were expressed only in HIV-1 infected individuals but not in those who were seronegative, which indicates that neopterin concentrations are independent of any certain T-cell phenotype. In addition, the correlations between neopterin concentrations and T-cell findings in anti-HIV-1 seropositive individuals are significant but by no means perfect. This might reflect certain differences between the underlying mechanisms; for example, T-cell counts are representative only for circulating blood, whereas neopterin is a marker for the immune status of the whole body.

Neopterin concentrations differed significantly between those negative and positive for anti-HIV-1, and our data indicate neopterin to be the most sensitive marker of those immunological alterations most likely caused by HIV-1 in seropositive persons. This may suggest that neopterin concentrations tend to increase earlier in the course of HIV infection, before effects on T-cell subpopulations become detectable. This assumption is supported by recent data of
others showing that higher neopterin concentrations pre-
sage subsequent loss of CD4+ T-cells in individuals infected
with HIV-1 (6).

The main application of nonspecific tests in HIV-1 infec-
tion is to monitor infected individuals and possibly to predict
the further course of disease. For this purpose sensitive
markers of cellular immunity are considered to reflect
indirectly the effects of HIV. The high sensitivity of neo-
terin concentrations looks promising. Moreover, prelimi-
inary results showed that neopterin concentrations are use-
ful in predicting the clinical course of individuals infected
with HIV-1. That is, asymptomatic subjects with higher
concentrations of neopterin were found to be more likely to
develop AIDS-related complex within the two years of the
study period (7).

The determination of neopterin is easily performed in
small amounts of urine or serum (8, 9). The test does not
introduce any burden for the patients and thus allows
frequent monitoring of patients during therapeutic studies.
Samples can be shipped and stored without pretreatment.

We conclude that neopterin measurement may be a
reasonable addition to those tests now used for the immune
monitoring of HIV-1–infected subjects. Further studies are
under way to investigate the predictive value of neopterin
concentrations in comparison with T-cell subset data.

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