Serum kynurenine-to-tryptophan ratio increases with progressive disease in HIV-infected patients

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An alternative pathway of Trp metabolism involves the conversion of Trp to kynurenine by indoleamine-2,3-dioxygenase, which leads to synthesis of the neurotoxin, quinolinic acid. This study explores the relationship of indoleamine-2,3-dioxygenase activity with stages of HIV infection. Sera from 206 HIV-positive and 72 seronegative subjects were analyzed for Trp and kynurenine. The kynurenine-to-Trp (KT) ratio was calculated. The mean KT ratio of seronegative controls was 36.6 ± 10.9, and the median ratio was 34.9. The upper limit of the seronegative KT ratio, defined as mean + 2 SD, was 58.4. Patients with HIV infection showed a reciprocal relationship between the KT ratio, the CD4 count, and the stage of the disease. The median KT ratios for asymptomatic and AIDS patients were 50.5 and 117.0, respectively. This study shows that the serum Trp concentration is markedly decreased and that the kynurenine concentration is increased with immune stimulation in HIV infection. This may lead to changes in quinolinic acid and explain some of the pathogenesis of AIDS dementia.

Infection with HIV is associated with a range of metabolic changes (1) that may contribute to the pathophysiology of the disease. Trp is an essential amino acid and precursor of serotonin. Trp is also connected to kynurenine (KYN)4 by an alternative pathway involving the enzyme indoleamine-2,3-dioxygenase (IDO). This is the first step in the synthesis of quinolinic acid and is rate limiting. Quinolinic acid is an agonist of N-methyl-d-aspartate-type excitatory receptors in the brain (2) and is considered one possible mediator of neurological dysfunction in AIDS (3).

Evidence indicates that the alternative Trp pathway is activated by immune stimulation. Activation of IDO by interferon-γ (4) is shown in several cell types as well as in the brain and lung tissue of macaque monkeys infected with simian immune deficiency virus (5). In human macrophages, conversion of Trp into quinolinic acid increases under interferon-γ stimulation (6). Decreased serum Trp correlates with markers of immune activation, such as serum neopterin concentrations (7).

We postulate that the alternative Trp pathway is stimulated by the progression of HIV disease, thus increasing the production of the neurotoxin, quinolinic acid. This may be a possible explanation for the increasing neurological damage in HIV disease as immune suppression advances. An increase in the serum KYN-to-Trp (KT) ratio would indicate activation of IDO. The present study explores the relationship of IDO activity with various stages of HIV infection.

Subjects and Methods

Patients
The study included 206 patients divided into two groups. Retrospective cohort. Seventy-five of the HIV-positive patients were from St. Mary’s Hospital, London, where they were part of a neuropathy study (8). The majority of them had either peripheral neuropathy (PN) or AIDS-related dementia (ARD) or had both. Sera taken from these patients between January 1989 and January 1993 and stored at −20 °C were available. Although detailed clinical endpoint data were available in this group, only 37 of them had data on CD4 lymphocyte counts, because this was not part of a routine clinical practice at the time.

Thirty-three patients were also included from Heartlands and General Hospitals, Birmingham, in whom clin-
tical endpoint data, CD4 counts, and stored sera (at −20 °C) were available.

Precise demographic data on this cohort were not available, but the overwhelming majority of the patients were men who had had sex with men (personal communication, J.B. Winer).

**Prospective cohort.** Ninety-eight patients were recruited prospectively between May 1992 and December 1995 from two dedicated outpatient clinics in Birmingham (The General Hospital and Heartlands Hospital), UK. Of these patients, 84 were Caucasian, 10 were Afro-Caribbean, and 4 were Asian. There were 3 women and 95 men; 87 patients were men who had had sex with men, 7 patients came from sub-Saharan Africa, and 4 patients had acquired the HIV infection heterosexually. There were no i.v. drug users in this cohort. The mean age was 34.9 years, and the range was 21–71 years.

Patients were seen at a mean interval of 196 days (6.5 months), and venous blood was collected. The mean follow-up was 240.5 days (range 24–978 days). Patients were questioned about neurological symptoms and received a standardized neurological examination at each visit. Neurological symptom and disability scores (9) were used to assess the extent of any neuropathic symptoms. Patients who scored ≥2 on the symptom score and ≥6 on the disability score were considered to have a neuropathy. Clinical endpoint data, including AIDS-defining events, evidence of clinically apparent PN, ARD, and death, were collected until August 31, 1996.

During the course of the study, 53 patients took zidovudine (AZT) and 23 took didanosine or zalcitabine at some time.

**CONTROLS**

Stored sera from 72 controls were obtained from male patients who attended the Genitourinary Medicine Clinic at Birmingham General Hospital for unrelated conditions and also had a negative HIV antibody test.

Informed consent was obtained from all control subjects and patients. The study had obtained the local ethical committee approval.

Unfortunately, no data on food intake or the relationship of blood samples and food ingestion in the retrospective cohort and the control subjects were available. Most blood samples from the prospective cohort were collected at midmorning, 2–4 h after breakfast.

**SAMPLE COLLECTION AND STORAGE**

Plasma samples were collected from the prospective cohort into heparin-containing tubes. The tubes were centrifuged at 2000g at 4 °C for 10 min, and the supernatants were collected within 24 h of venipuncture. The supernatants were stored in −20 °C freezer. The stored samples from the retrospective cohort were sera stored in a −20 °C freezer. However, the time between venipuncture and supernatant collection and storage is unknown.

**SAMPLE PREPARATION**

Serum concentrations of Trp and KYN were measured using isocratic, reversed-phase HPLC (Jasco) and spectrophotometric detection (Pharmacia Biotech). The analytical column consisted of a 15 cm × 4.6 mm i.d. column packed with 5-μm particles of Shanden Hypersil octadecylsilane. A cartridge guard column (10 mm × 3.2 mm o.d.) containing the same material as the analytical column was used. Column temperature was maintained by a column block heater (Model TC-970, HPLC Technology). A chromate PC data system (PU 4820, Philips Scientific) was used for data collection and handling.

For Trp measurement, 125 μL of specimen was added to 125 μL of 4.5% perchloric acid. After the mixture stood for 10 min at room temperature and was centrifuged at 4000g for 30 min, the clear supernatant was removed for analysis. Mobile phase was prepared with 20 mL of acetonitrile diluted in 1 L of distilled water. Concentrated perchloric acid (70%, 0.62 mL) was added to produce a pH of 2.0, followed by the addition of 10 μL of octylaniline. The sample (20 μL) was then injected onto the column. The column temperature was 31 °C; the flow rate was increased from 0.1 mL/min to 3.0 mL/min over 3 min and maintained at 3 mL/min for an additional 9 min, then decreased to 0.1 mL/min over the next 3 min. Ultraviolet detection was at 215 nm. The retention time for Trp was 8.3 min (10).

For KYN detection, 270 μL of specimen was mixed with 30 μL of perchloric acid. This was placed on ice for 15 min and centrifuged at 4000g for 30 min. The clear supernatant was removed for analysis. HPLC was carried out at a flow rate of 1.0 mL/min with a mobile phase prepared by mixing 5 mL of acetonitrile with 235 mL of 0.1 mol/L acetic acid, 0.1 mol/L ammonium acetate, pH 4.65. The injection volume was 20 μL. The absorbance was at 365 nm. The retention time for KYN was 5.5 min (11).

The standard Trp and KYN were supplied by Sigma Chemicals.

Concentrations were expressed in μmol/L. The KT ratio was calculated as an index of IDO activity. In the interest of clarity, the ratio is multiplied by 1000 and expressed as the KT ratio in this study.

**STATISTICAL METHODS**

The Minitab Computer software package (Ver. 11) was used for statistical analysis. Where variables have a distribution skewed to the left, possibly due to the relatively small sample size, a nonparametric method (Mann–Whitney U-test) was used to compare groups.

**Results**

Of the cohort of 206 HIV-positive patients, 102 patients (49.3%) either entered the study with AIDS-defining illnesses or developed AIDS-defining illnesses during the course of the study; 41 patients had clinically evident PN and 25 had ARD, including 9 patients who had both. One
The mean KT ratio was also evaluated 18, 12, 6, 3, and 1 month before and 6, 12, and 18 months after patients’ AIDS-defining event, provided that no further AIDS-defining illnesses occurred during this time. The data are presented in Fig. 1. The KT ratio was significantly lower 2–3 months before and 6 months or more after the diagnosis of AIDS compared with levels at the time of the diagnosis ($P < 0.05$).

Life table analysis shows that an abnormal KT ratio (i.e., $>58.4$) is associated with significantly earlier development of the first AIDS-defining event ($P < 0.001$) (Fig. 2).

CD4 counts were available in 116 patients. The mean CD4 count of these patients on entry into the study was $287/\text{mm}^3$ (range, 1–1300/\text{mm}^3); at the end of the study, the mean CD4 count was $229/\text{mm}^3$ (range 0–890/\text{mm}^3). Data comparing the Trp and KYN concentrations and the KT ratio with the CD4 count are also presented in Table 1. Trp concentrations decreased and KYN concentrations increased with decreasing CD4 counts, giving rise to a marked increase of the KT ratio with worsening immune function.

Patients from the prospective cohort had detailed data on antiretroviral therapy collected. Analyses were done on 15 patients who had KT ratio data available before and within 12 months after starting AZT. The median ratio was 67.3 before and 59.1 after AZT, which is not statistically significant ($P > 0.05$).

### Discussion

Under normal circumstances, the IDO enzyme appears to be tightly controlled. This is reflected by the very narrow 95% confidence interval for the control group KT ratio (32.4–36.9).

In this study, an increase in the KT ratio associated with HIV infection is caused by decreases in Trp concentrations and increases in KYN concentrations in the serum. This is consistent with Trp degradation through the KYN pathways and reflects activation of the IDO enzyme in systemic tissues. Previous in vitro studies have already shown that measurement of Trp metabolites (including KYN) is a very sensitive indicator of Trp degradation induced by interferons (4).

Previous studies have also shown decreased serum Trp concentrations in HIV disease (1, 7). Substantial correlation has also been found between serum interferon-γ concentrations and Trp degradation in HIV-positive patients (12, 13). Our data supports these previous studies and confirms the role of immune stimulation in Trp degradation. We found a substantial reciprocal relationship between the CD4 count and the KT ratio, which is markedly increased around the time of an AIDS-defining illness (see Table 1). Even in patients who are asymptomatic, the KT ratio was increased compared with the control group, though to a lesser extent. When the values of the KT ratio before and after an AIDS-defining event were compared, it appears that the KT ratio increases occur 1–2 months before the diagnosis of AIDS and reach their peak

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**Table 1. Median and 95% confidence interval for Trp (\(\mu \text{mol}/\text{L}\)), KYN (\(\mu \text{mol}/\text{L}\)), and KT ratio in different patient groups and correlation with CD4 counts.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Trp,(^a) (\mu \text{mol} /\text{L})</th>
<th>KYN,(^a) (\mu \text{mol} /\text{L})</th>
<th>KT ratio,(^a) x 1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal(^b)</td>
<td>56.3 (53.3, 58.0)</td>
<td>1.98 (1.86, 2.10)</td>
<td>34.9 (32.4, 36.9)</td>
</tr>
<tr>
<td>(A) n = 72</td>
<td>(50.1, 55.7)</td>
<td>(2.55, 2.79)</td>
<td>(50.5, 55.5)</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>(47.8, 53.7)</td>
<td>(2.29, 2.80)</td>
<td>(44.9, 54.5)</td>
</tr>
<tr>
<td>AIDS</td>
<td>33.2(^c,d)</td>
<td>3.98(^e,d)</td>
<td>119.9(^e,d)</td>
</tr>
<tr>
<td>(C) n = 12</td>
<td>(29.0, 49.5)</td>
<td>(3.49, 4.44)</td>
<td>(75.1, 143.4)</td>
</tr>
<tr>
<td>PN/ARD</td>
<td>48.6(^c,d)</td>
<td>4.17(^e,d)</td>
<td>87.8(^e,d)</td>
</tr>
<tr>
<td>(D) n = 10</td>
<td>(33.9, 55.0)</td>
<td>(3.44, 4.58)</td>
<td>(74.2, 126.1)</td>
</tr>
<tr>
<td>Death</td>
<td>41.7(^c,d)</td>
<td>3.13(^e,d)</td>
<td>79.5(^e,d)</td>
</tr>
<tr>
<td>(E) n = 9</td>
<td>(30.3, 44.1)</td>
<td>(2.55, 4.02)</td>
<td>(47.4, 104.4)</td>
</tr>
<tr>
<td>CD4 count</td>
<td>55.1(^c)</td>
<td>2.50(^f)</td>
<td>49.7(^f)</td>
</tr>
<tr>
<td>&gt;500/\text{mm}³</td>
<td>(48.0, 59.0)</td>
<td>(2.3, 2.71)</td>
<td>(45.0, 52.1)</td>
</tr>
<tr>
<td>n = 34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 count</td>
<td>44.0(^f)</td>
<td>3.44(^f)</td>
<td>59.0(^f)</td>
</tr>
<tr>
<td>200–500/\text{mm}³</td>
<td>(41.0, 46.7)</td>
<td>(3.14, 3.61)</td>
<td>(53.9, 63.40)</td>
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<tr>
<td>n = 89</td>
<td></td>
<td></td>
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<tr>
<td>CD4 count</td>
<td>43.8(^f)</td>
<td>3.54(^f)</td>
<td>76.8(^f)</td>
</tr>
<tr>
<td>&lt;200/\text{mm}³</td>
<td>(41.4, 45.2)</td>
<td>(3.3, 3.7)</td>
<td>(73.5, 82.5)</td>
</tr>
<tr>
<td>n = 132</td>
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</table>

\(^a\) Value in parentheses designates 95% confidence interval.

\(^b\) Letter in parentheses designates group.

\(^c\) $P < 0.01$ compared with normal controls.

\(^d\) $P < 0.01$ compared with asymptomatic patients.

\(^e\) $P < 0.05$ compared with normal controls.

\(^f\) All concentrations are significantly different from each other ($P < 0.01$); no statistically significant differences among AIDS, PN/ARD, and death group (Mann–Whitney U-test).
within 30 days of the diagnosis. The ratio decreased markedly within 6 months after the AIDS diagnosis to values comparable to the asymptomatic stage. In the absence of further AIDS-defining events, it remained at this relatively low value for at least 12 months.

Patients who developed PN/ARD also had a markedly increased KT ratio compared with asymptomatics or controls (Table 1). Although our study did not demonstrate any substantial difference between the patients with PN/ARD and those with AIDS but without neurological complications, this is mainly because all but one of the patients with PN/ARD studied also had AIDS.

In contrast to one previous study (14), we were unable to demonstrate any changes in the KT ratio after 1–12 months of AZT therapy. However, our study was not designed to assess antiretroviral effect, and the numbers are too small to make meaningful conclusions.

One can postulate that the consequences of this observed increase in IDO activity with progressive HIV disease may explain some of the pathogenesis of AIDS. Depletion of Trp may impair protein synthesis, which in turn can contribute to weight loss (15), diarrhea, and the dementia seen in HIV disease [reviewed by Brown et al. (16)].
Decreased availability of Trp may also decrease production of the neurotransmitter serotonin, promoting affective disorders and contributing to neuropsychiatric diseases in HIV infection (17). Evidence indicates that an increase in Trp intake may decrease psychological distress (18). More importantly, when the KYN concentration is increased through immune activation, production of its neuroactive metabolites, such as quinolinic acid, is stimulated. Serum concentrations of Trp and KYN correlate with those in the cerebrospinal fluid (13, 14, 17, 19, 20), and increased IDO activity was found in the brain of retrovirus-infected macaques (5) and AIDS dementia patients (21). Increased quinolinic acid concentrations have been demonstrated in the cerebrospinal fluid and brain tissue of patients and nonhuman primates with AIDS (3) and simian AIDS (22). These may be important mediators of neurological dysfunction (23). If this hypothesis is correct, strategies that can decrease neuroactive KYN metabolites or attenuate their effects may offer new therapeutic approaches for HIV-related neurological complications.

We thank Sue Drake and her team from Birmingham Heartlands Hospital, Jonathan Weber from St. Mary’s Hospital, London, and Tony Pinching from St. Bartholomew’s Hospital, London, for their support in this project. This study was funded in part by the Medical Research Endowment Fund, University of Birmingham, UK (grant no. F08691)

References