fluid intake is little explored, but will probably be useful. The corollary of this, a possible increase in obsessionality in a vulnerable personality, must also be considered, as it must in the management of end-stage renal failure when the mode of treatment is decided. With these provisions, the scope for self-testing appears very wide, and we look forward to further developments in the technology upon which this depends.

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β2-Microglobulin as a Prognostic Marker for Development of AIDS

To the Editor:

Acquired immunodeficiency syndrome (AIDS) is the final culmination of a disease that apparently exists in other forms such as AIDS-related complex or lymphadenopathy syndrome, and in less-manifested states, including the carrier state. Blood-donor screening by use of an antibody to the presumed causative virus (HTLV-III/LAV) has begun. Such testing for the virus only detects exposure to it, not the presence of, or the prognosis for, development of disease. Also, the antibody test for HTLV-III sometimes fails to detect the presence of the virus (1). Thus, another test is needed that will allow a more quantitative assessment of the immune system’s response to HTLV-III exposure.

Of other markers that could be used for quantification, the one that has received the most attention has been β2-microglobulin (βm) (2–9), a small (M, 11 800) polypeptide that is the light chain of human leukocyte antigen and is part of T- and B-cell receptors. The concentration of βm in serum increases in lymphoproliferative disorders as the immune dysfunction progresses. In 1983, we reported (5) that 29 of the 31 AIDS patients tested had above-normal concentrations of βm, and so did five out of 11 normal homosexual men. Zolla-Pazner et al. (8) found that all of their 39 patients with AIDS or suspected AIDS had above-normal βm concentrations, even two years before its clinical diagnosis. In another study (5), increased βm concentrations were found not only in the sera but also in the urine of AIDS patients. We have measured βm in 210 different AIDS patients, finding increased concentrations in 194 of them, a percentage remarkably similar to that observed for HTLV-III seen in AIDS cases (10). Moreover, βm concentrations were 10- to 100-fold higher in factor VIII preparations made in the U.S. as compared with those prepared in England (11), which is in keeping with the transmission of AIDS to hemophiliacs that has occurred from U.S.-prepared factor VIII concentrates.

We now have used cross-radioimmunoelectrophoresis, a technique originally developed to study lymphocyte-associated βm (12), to test sera from both normal persons and AIDS patients. When normal sera are used, only one peak in the βm region is observed (Figure 1, top). AIDS sera show, in addition to this normal peak, another faster-migrating peak termed the α-electrophoretic form of βm (Figure 1, bottom). The following tabulation summarizes our findings. The α-electrophoretic form of βm was detected in all the patients listed, including those with normal βm values (noted with an asterisk).

<table>
<thead>
<tr>
<th>Disease states</th>
<th>No. of patients studied</th>
<th>Total βm conc., mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS/Kaposi’s sarcoma (KS)</td>
<td>14</td>
<td>all &gt;3.5</td>
</tr>
<tr>
<td>Lymphadenopathy only</td>
<td>2</td>
<td>all &gt;3.0</td>
</tr>
<tr>
<td>HTLV-III-positive</td>
<td>3</td>
<td>all &gt;3.0</td>
</tr>
<tr>
<td>AIDS/KS</td>
<td>5*</td>
<td>all &lt;1.7</td>
</tr>
<tr>
<td>HTLV-III-negative</td>
<td>1*</td>
<td>1.9</td>
</tr>
</tbody>
</table>

We next measured βm (total and α-electrophoretic form) in HTLV-III-test ed AIDS sera. In this double-blind study, of 43 HTLV-III-positive AIDS patients, 38 had above-normal concentrations of total βm. Cross-radioimmunoelectrophoresis indicated the presence of the α-electrophoretic form of βm in all of the remaining five patients. But more importantly, the α-electrophoretic form of βm was found in an AIDS patient who was HTLV-III-negative. Additional data (manuscript in preparation) on the α-electrophoretic form of βm indicates that this analyte detects the carrier state of AIDS as reflected by transmission of AIDS from mother to infant. This suggests that the faster migrating peak confirms disease development.

The cause of this type of heterogeneity, resulting in two electrophoretic forms, is not known in humans; but it has been demonstrated in mice that such heterogeneity could result from a single amino-acid substitution (13). It is also possible that it could result from the cleavage of part of the molecule, or from the binding of another substance that alters its electrophoretic mobility. Other studies (14), as well as those we are conducting, show that this phenomenon occurs in other immune diseases and may reflect a common response of the body to immune-disease progression. This seems reasonable because βm has been conserved throughout evolution and is one of the building blocks of the immune system.

We conclude that βm (total and α-electrophoretic form) may serve as a useful prognostic marker for the development of AIDS. Accordingly, this analyte can be used adjunctively to the HTLV-III-antibody testing already initiated. This, of course, is a preliminary study that needs further confirmation on a more elaborate scale.

References


Fig. 1. Autoradiograph of crossed-radioimmunoelectrophoresis of serum from a normal, healthy control (top) and patient with AIDS/Kaposi’s sarcoma (bottom).

"a" denotes the presence of α-electrophoretic form of βm in AIDS/Kaposi’s sarcoma patients.

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Direct Radioluminoassay of Melatonin in Saliva

To the Editor:

Investigations into use of the pineal gland hormone, melatonin, as a putative biological marker for the affective disorders (1,2) and for pinealoma (3,4) have generally relied on results from radioimmunoassay. The advent of sensitive, specific methods for estimating melatonin in plasma has done much to facilitate clinical research, but there are practical difficulties associated with taking samples of blood at midnight or early morning for melatonin assay. The current assay on assay of hormones in saliva (5), which obviates such difficulties, has led to our developing such an assay for melatonin that involves no extraction with solvents, which—with use of a new tracer with high specific activity—we have used in investigating the relationship between plasma and saliva (Miles et al., in preparation).

We used N-[(trihydroxymethyl)methyl]glycine ("Tricine"), activated charcoal (untreated powder), Dextran T70, and melatonin from Sigma Chemical Co., St. Louis, MO 63178; [3H]melatonin (spec. acty., 81 KCl/mol) from Amerham International plc, Amerham, Bucks, U.K.; antisemur (batch no. 704/6483) from Guildhay Antisaera, University of Surrey, U.K. All other commonly used laboratory reagents were of "Analar" grade, from British Drug Houses, Poole, Dorset, U.K. Disposable plastic apparatus was used wherever possible and all glassware was acid-washed and rinsed in methanol before use.

Saliva, sampled without stimulation of its production, was collected into disposable plastic tubes and stored at −20°C for at least 24 h. After thawing, the samples were centrifuged at 2500 × g for 15 min to remove solids. We assay 500-μL duplicate aliquots, comparing results directly against a standard curve prepared in assay buffer (0.1 mol/L "Tricine," pH 7.4, containing 9 g of NaCl and 1 g of gelatin per liter). The standard curve covers the range 0 to 100 pg per tube. We used the sequence 2, 2.5, 5.0, 12.5, 25.0, 50.0, and 100 pg per tube.

The procedure: To 500 μL of saliva and standard add 150 μL of antiserum (initial dilution, 2000-fold in assay buffer). Vortex-mix the contents of the tubes and incubate them at room temperature for 45 min. Then add 50 μL of buffer containing 3000 cpm of [3H]melatonin to all tubes, vortex-mix, and incubate at 4°C for 20 h. Perform all subsequent procedures at 4°C. Separate the antibody-bound melatonin from the free fraction by incubating the mixture with 450 μL of dextrancoated charcoal suspension (10 g of charcoal, 0.1 g of Dextran T70 per 500 mL of assay buffer, stirring the mixture at 4°C for 10 min. Then centrifuge for 10 min at 1700 × g. Transfer 600 μL of the supernate to 4 mL of toluene based scintillant, shake the mixture for at least 30 min, and equilibrate in a scintillation counter before counting the radioactivity. Using the standard curve, calculate the melatonin concentrations in the samples. Within- and between-assay CVs for 12 replicate standard curves showed the precision of the assay system to be satisfactory (Table 1). Analytical recovery of hormone, measured at three different concentrations, ranged from 97.9 to 103.5% with an assay sensitivity (defined as the least amount of hormone distinguishable from zero) of 4 pg per tube. Nonspecific binding of antibody was <2%. The antibody characteristics have been characterized by the University of Surrey, U.K. The only measurable cross reactivity (expressed relative to the quantity of melatonin that displaces 50% antibody-bound [3H]melatonin) was found to be from N-acetyltysine (0.91%), 6-hydroxymelatonin (0.33%), and N-acetyltyslypropan (0.22%). All other structurally related compounds that we tested cross reacted by <0.06%.

In a recent study of the nocturnal profile of salivary melatonin (Miles et al., in preparation) we have shown that the secretory pattern of melatonin in plasma is mirrored in saliva, except that the mean concentration is 24% that in the corresponding plasma. Results for paired plasma and saliva samples, taken hourly from six healthy volunteer subjects between 20:00 and 08:00 hours, correlated very well (r = 0.971; p < 0.001). Hormone secretion in these volunteers was greatest at 01:00 hours, the melatonin concentration in plasma being 94.9 (SEM 15.3) ng/L and the corresponding value for saliva being 24.4 (SEM 3.1) ng/L. At 08:00 hours, the concentration in plasma had declined to 33.9 (SEM 5.9) ng/L with 9.7 (SEM 1.8) ng/L being detectable in saliva. Melatonin reportedly is about 70% bound to plasma albumin, and the salivary fraction may thus represent the "free" (i.e., unbound) hormone. Differences in the plasma-saliva ratio for melatonin seen at some points in compared overnight plasma and salivary hormone profiles were suggested by results of a subsequent study to be ascribable to a dilution effect, i.e., changes in salivary flow rate. Such differences were minimized if saliva was collected without any stimulation of its production.

We anticipate that assay of melato-

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Table 1. Precision of the Assay for Salivary Melatonin, As Assessed from Data on Quality-Control Samples

<table>
<thead>
<tr>
<th>Pool</th>
<th>Mean, ng/L</th>
<th>SD, ng/L</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.7</td>
<td>1.10</td>
<td>5.31</td>
</tr>
<tr>
<td>2</td>
<td>64.8</td>
<td>4.11</td>
<td>6.34</td>
</tr>
<tr>
<td>3</td>
<td>140.7</td>
<td>3.09</td>
<td>2.19</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Pool</th>
<th>Mean, ng/L</th>
<th>SD, ng/L</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21.0</td>
<td>2.71</td>
<td>12.9</td>
</tr>
<tr>
<td>2</td>
<td>71.3</td>
<td>2.21</td>
<td>3.1</td>
</tr>
<tr>
<td>3</td>
<td>138.3</td>
<td>3.72</td>
<td>2.68</td>
</tr>
</tbody>
</table>