Severe Deficiency of 1,25-Dihydroxyvitamin D₃ in Human Immunodeficiency Virus Infection: Association with Immunological Hyperactivity and Only Minor Changes in Calcium Homeostasis

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ABSTRACT

The serum level of 1,25-dihydroxyvitamin D₃ [1,25-(OH)₂D₃], the biologically most potent metabolite of vitamin D₃, is tightly regulated within narrow limits in human healthy adults. 1,25-(OH)₂D₃ deficiency is rare and is associated with disturbances in calcium and bone metabolism. We have previously reported a marked decrease in serum levels of 1,25-(OH)₂D in human immunodeficiency virus (HIV)-infected patients. The present study was designed to further examine the causes and consequences of severe 1,25-(OH)₂D deficiency in these patients. The design was a prospective cohort study. Fifty-four HIV-infected patients clinically classified according to the revised criteria from Centers for Disease Control and Prevention and healthy controls were studied. Parameters related to vitamin D and calcium metabolism as well as immunological and nutritional status were determined. Twenty-nine of the patients (54%) had serum levels of 1,25-(OH)₂D below the lower reference limit, and 18 of these had undetectable levels. In contrast, HIV-infected patients had normal serum levels of 25-hydroxyvitamin D and vitamin D-binding protein. HIV-infected patients as a group had modestly depressed serum calcium and PTH levels. There were, however, no correlations between these parameters and serum levels of 1,25-(OH)₂D. There were no differences in serum calcium or PTH levels or nutritional status when patients with severe 1,25-(OH)₂D deficiency were compared to other patients, but patients with undetectable 1,25-(OH)₂D had significantly elevated serum phosphate levels. Furthermore, patients with undetectable 1,25-(OH)₂D levels were characterized by advanced clinical HIV infection, low CD4⁺ lymphocyte counts, and high serum levels of tumor necrosis factor-α (TNFα).

We conclude that inadequate 1α-hydroxylation of 25-hydroxyvitamin D seems to be the most likely cause of 1,25-(OH)₂D deficiency in HIV-infected patients, possibly induced by an inhibitory effect of TNFα. The low 1,25-(OH)₂D and high TNFα levels observed may impair the immune response in HIV-infected patients both independently and in combination and may represent an important feature of the pathogenesis of HIV-related immunodeficiency. Markedly depressed 1,25-(OH)₂D serum levels are also present in certain other disorders characterized by immunological hyperactivity. Thus, the findings in the present study may not only represent a previously unrecognized immune-mediated mechanism for induction of 1,25-(OH)₂D deficiency in human disease, but may also reflect the importance of adequate serum levels of 1,25-(OH)₂D for satisfactory performance of the immune system in man. (J Clin Endocrinol Metab 83: 3832–3838, 1998)

THE SERUM level of 1,25-dihydroxyvitamin D₃ [1,25-(OH)₂D₃], the biologically most potent metabolite of vitamin D₃, is tightly regulated within narrow limits in human nondiseased adults (1, 2) and may be normal even in severe vitamin D-deficient rickets when levels of its precursor 25-hydroxyvitamin D₃ (25D) are very low or even undetectable (3). 1,25-(OH)₂D₃ deficiency is rare, but is present in disorders with impaired renal 1α-hydroxylation of 25D, such as hereditary defects of this enzyme (vitamin D-dependent rickets type I) and severe renal failure (3–5), leading to disturbances in calcium and bone metabolism.

Until the advent of antibiotics, vitamin D was used to treat mycobacterial infections (6, 7), and vitamin D deficiency has been associated with impaired cellular immunity (8, 9). More recently, a substantial amount of experimental data from in vitro studies has established 1,25-(OH)₂D₃ as a potent modulator of cells in the immune system (2, 10, 11).

We have previously, for the first time, reported a marked decrease in serum levels of 1,25-(OH)₂D in human immunodeficiency virus (HIV)-infected patients correlating with the degree of immunodeficiency and survival. Particularly low levels were seen in acquired immunodeficiency syndrome (AIDS) patients with ongoing Mycobacterium avium complex (MAC) infection (12, 13). The low 1,25-(OH)₂D levels appeared not to be related to deficiency of 25D, and the reason for the low levels in HIV-infected patients remains unclear.

The present study was designed to further examine the reasons for and the metabolic consequences of the low 1,25-(OH)₂D serum levels in HIV-infected patients. As 1,25-(OH)₂D has a central role in the complex interactions of calcium, phosphate, PTH, and calcitonin in calcium homeostasis, we wanted, in particular, to examine the conse-
quences of severe 1,25-(OH)\textsubscript{2}D deficiency on these parameters.

**Subjects and Methods**

**Patients and controls**

Fifty-four consecutively recruited HIV-infected patients (44 males and 10 females; median age, 36 yr; range, 16–64 yr) were included in the study and were classified according to the revised criteria from Centers for Disease Control and Prevention (CDC; 1992) as asymptomatic HIV infection (CDC group A; n = 15), symptomatic non-AIDS HIV infection (CDC group B; n = 12), and AIDS (CDC group C; n = 27). Within a time span of 4 weeks (from 3 weeks before to 1 week after blood sampling), 16 patients had an ongoing clinical event (chronic symptomatic cytomegalovirus-infection, n = 4; chronic MAC infection, n = 3; chronic symptomatic hepatitis C virus infection, n = 1; *Candida* esophagitis, n = 1; Kaposi's sarcoma with visceral involvement, n = 2; bacterial pneumonia, n = 3; AIDS dementia complex, n = 1; cryptococcal meningitis, n = 1). Twenty patients had diarrhea (>2 loose stools/day) persisting more than 30 days and weight loss of more than 10% of body weight or both; 4 of these patients were classified as having wasting syndrome (CDC, 1992).

None of the patients was abusing drugs or alcohol at the time of the study, and all except four had serum creatinine levels within the normal range.

Controls in the study were 20 sex- and age-matched healthy blood donors for tumor necrosis factor-α (TNFα), and CD4 and CD8 lymphocytes. For the other parameters, reference values in the laboratory were used.

**Blood-sampling protocol**

Blood samples were drawn between 0800–1000 h after an overnight fast into sterile vacuum blood collection tubes without any additives. The tubes were immediately immersed in ice water and allowed to clot for less than 1 h before centrifugation. The serum samples were stored at −70° C until analysis and were frozen and thawed only once.

**Quantification of serum levels of vitamin D metabolites**

Serum levels of 1,25-(OH)\textsubscript{2}D and 25D were analyzed by two different methods. First, 34 samples were analyzed with RIAs obtained from Nichols Institute Diagnostics (Wijchen, The Netherlands), as described previously (12). Then, 24 samples were analyzed using methods established at The Hormone Laboratory, Aker University Hospital (Oslo, Norway) (14). Briefly, vitamin D\textsubscript{3} metabolites were measured after extraction of serum with diethyl ether and chromatographic separation. As quantification of 1,25-(OH)\textsubscript{2}D requires 2 ml serum, only four samples were analyzed at both laboratories, two with undetectable 1,25-(OH)\textsubscript{2}D levels and two with serum levels in the normal range. Samples with undetectable serum levels were undetectable in both laboratories; the differences between the other two samples were 11% and 18%, respectively. Samples from the two laboratories were first analyzed separately to look for correlations with clinical and laboratory parameters. When they showed similar patterns, the results from the two laboratories were pooled for further statistical analyses.

Serum levels of vitamin D-binding protein (DBP) were measured by standard methods using rocket immunoelectrophoresis (15).

**Quantification of serum levels of PTH**

Serum levels of intact PTH were quantified using a solid phase, two-site chemiluminescent enzyme immunoassay for use with the IMMULITE automated analyzer (Diagnostic Product Corp., Los Angeles, CA).

**Quantification of other biochemical parameters**

Serum levels of creatinine, albumin, iron, phosphate, and magnesium were analyzed in a Hitachi-917 autoanalyzer, and serum levels of ionized calcium were analyzed in a Hitachi-987 autoanalyzer (Hitachi Scientific Instruments, Tokyo, Japan), both with reagents from Boehringer Mannheim (Mannheim, Germany). Serum levels of prealbumin were quantified by nephelometry calibrated by commercial standards (Boehringer Mannheim). Serum cobalamin and folate in serum and erythrocytes were quantified by standard methods using RIAs from Diagnostic Products Corp. Serum levels of zinc were measured by atomic absorption spectrophotometry (Perkin-Elmer, Norwalk, CT), and calcitonin was determined by RIAs from CIS-Bio International (Gif-sur-Yvette, France).

**Quantification of TNFα**

TNFα in serum samples was determined by an enzyme immunoassay from Medgenix (Fleurus, Belgium) as previously described (13).

**Determination of T lymphocyte subsets**

The numbers of CD4\textsuperscript{+} and CD8\textsuperscript{+} lymphocytes in peripheral blood were determined by immunomagnetic quantification as described previously (12).

**Statistical analysis**

For comparison of two groups, Fisher's exact test was used when analyzing table frequencies, a two-tailed Mann-Whitney U test was used when dealing with continuous data, and the Kruskal-Wallis ANOVA was used for comparison of three or more groups. Coefficients of correlation (r) were calculated by the Spearman rank test. For several of the clinical chemistry analyses no control group was available. Instead, a Wilcoxon test for one sample (signed rank sum) was used for the difference d = X - m (variable in clinical group) - m (median of the reference interval). When dealing with symmetrical distributions, m was calculated as the arithmetic mean of lower and upper reference intervals. For PTH and S- and E-folate, the mean was calculated for log-transformed data, and m was the corresponding retransformed value. The same procedure was applied to the calculation of quartiles. Data are given as the median and 25–75th percentiles unless otherwise stated.

**Results**

**Vitamin D metabolites**

In accordance with our previous results, we found that HIV-infected patients had significantly depressed serum levels of 1,25-(OH)\textsubscript{2}D compared to controls (Table 1). Twenty-nine patients had serum levels below the normal range, and 18 of these had undetectable serum levels of 1,25-(OH)\textsubscript{2}D. This marked decrease in serum levels of 1,25-(OH)\textsubscript{2}D was confirmed by two different methods for analyzing 1,25-(OH)\textsubscript{2}D levels (see Materials and Methods).

Serum levels of both 25D and DBP were within the normal ranges, and there were no differences in serum levels between HIV-infected patients and controls (Table 1).

When patients with undetectable 1,25-(OH)\textsubscript{2}D levels were compared to other patients, there were no significant differences in levels of either 25D or DBP, although there was a slight reduction in DBP in patients with undetectable 1,25-(OH)\textsubscript{2}D levels (Fig. 1, C and D). However, even in these patients DBP levels were within the normal range.

**Diarrhea, weight loss, and malabsorption**

Twenty of the patients suffered weight loss and/or diarrhea, but the 1,25-(OH)\textsubscript{2}D levels in these patients were not significantly different from those in other HIV-infected patients (data not shown). The patients were screened for malabsorption and malnutrition by analysis of serum levels of albumin, prealbumin, iron, magnesium, zinc, folate, and vitamin B12 and folate levels in erythrocytes. Some of these
TABLE 1. Median and 25th and 75th percentiles of parameters related to vitamin D and calcium metabolism as well as immunological and nutritional status in HIV-infected patients (n = 54) compared to controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HIV-infected patients</th>
<th>Reference values</th>
<th>Statistical difference (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D-related parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,25-Dihydroxyvitamin D$_3$ (pmol/L)</td>
<td>48 (10°–82)</td>
<td>95 (80–110)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>25-Hydroxyvitamin D$_3$ (pmol/L)</td>
<td>70.1 (39.9–100)</td>
<td>70.0 (50–90)</td>
<td>NS</td>
</tr>
<tr>
<td>Vitamin D-binding protein (mg/L)</td>
<td>375 (325–420)</td>
<td>385 (339–431)</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium-related parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ionized calcium (mmol/L)</td>
<td>1.19 (1.13–1.24)</td>
<td>1.25 (1.23–1.27)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.10 (0.90–1.30)</td>
<td>1.15 (1.06–1.33)</td>
<td>NS</td>
</tr>
<tr>
<td>PTH (pmol/L)</td>
<td>2.00 (1.3–2.8)</td>
<td>2.69 (1.99–3.67)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Calcitonin (pmol/L)</td>
<td>2.10 (1.6–2.9)</td>
<td>&lt;4.5</td>
<td>NS</td>
</tr>
<tr>
<td>Renal function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>78 (73–93)</td>
<td>86 (62–110)</td>
<td>NS</td>
</tr>
<tr>
<td>Nutritional status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>36.0 (31.0–41.6)</td>
<td>42.7 (40–45)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Prealbumin (g/L)</td>
<td>0.21 (0.14–0.28)</td>
<td>0.30 (0.26–0.34)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Folate (serum; nmol/L)</td>
<td>12.5 (10.9–19)</td>
<td>11.0 (8.9–13.5)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Folate (erythrocytes; nmol/L)</td>
<td>545 (440–815)</td>
<td>765 (637–918)</td>
<td></td>
</tr>
<tr>
<td>Magnesium (mmol/L)</td>
<td>0.80 (0.80–0.90)</td>
<td>0.85 (0.80–0.90)</td>
<td>NS</td>
</tr>
<tr>
<td>Zinc (µmol/L)</td>
<td>12.0 (11.0–14.0)</td>
<td>13.0 (12.0–14.0)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Undetectable values registred as 10 pmol/L.

Variables were significantly decreased in the patient group compared with control values (Table 1). However, there were no correlations between parameters of malabsorption and malnutrition and 1,25-(OH)$_2$D levels among HIV-infected patients, and we found no significant differences in these parameters when patients with undetectable 1,25-(OH)$_2$D levels were compared (data not shown).

All four patients diagnosed as having wasting syndrome had undetectable 1,25-(OH)$_2$D levels.

**Serum levels of calcium, phosphate, PTH, and calcitonin**

As shown in Table 1, serum levels of ionized calcium and PTH were modestly, but significantly, decreased in HIV-infected patients compared to controls. However, there were no significant differences in calcium and PTH levels when patients with undetectable 1,25-(OH)$_2$D levels were compared to other patients (Fig. 1, A and E). There was a significant (P < 0.05) increase in serum phosphate levels when patients with undetectable 1,25-(OH)$_2$D levels were compared to patients with normal and low 1,25-(OH)$_2$D levels, but phosphate levels were still within the normal range (Fig. 1B). Serum levels of 1,25-(OH)$_2$D and phosphate in HIV-infected patients were inversely correlated (r = -0.49; P < 0.001).

Calcitonin levels were within the normal range (Table 1), and there were no significant differences in serum calcitonin between HIV-infected patients with undetectable 1,25-(OH)$_2$D serum levels and other patients (data not shown).

**Clinical manifestations**

As shown in Fig. 2, symptomatic HIV-infected patients (CDC groups B and C) had significantly lower serum levels of 1,25-(OH)$_2$D compared to controls (P < 0.05), with the lowest values in CDC group C. In CDC group C, eight severely immunodeficient patients (CD4$^+$ lymphocyte count range, 1–28 × 10$^6$ cells/L) had chronic, ongoing events; 6 of these patients (2 MAC, 3 CMV, and 1 hepatitis C) had undetectable 1,25-(OH)$_2$D levels, and 1 (MAC) had a serum level below the normal range.

**Immunological parameters**

When patients with undetectable 1,25-(OH)$_2$D levels were compared to other patients, CD4$^+$ lymphocyte counts were significantly (P < 0.01) lower in the former group (Fig. 1F). However, there was no strong correlation between 1,25-(OH)$_2$D and CD4$^+$ lymphocyte counts in the entire patient population (r = 0.26; P = 0.06).

CD8$^+$ lymphocyte counts were not significantly different in HIV-infected patients and controls (Table 1) and did not change significantly with different 1,25-(OH)$_2$D levels (Fig. 1G).

Sustained TNF$\alpha$ activation is a distinctive feature of the persistent immune activation seen in HIV infection (13), as also demonstrated in the present study (Table 1). Serum levels of TNF$\alpha$ were significantly higher in patients with undetectable 1,25-(OH)$_2$D (Fig. 1H), and there was a strong negative correlation (r = -0.55; P < 0.001) between serum levels of TNF$\alpha$ and 1,25-(OH)$_2$D in HIV-infected patients.

**Discussion**

Vitamin D from food or skin is hydroxylated in the liver to 25D and is further hydroxylated in the kidneys to the active metabolite 1,25-(OH)$_2$D, which is quickly metabolized to less potent metabolites. Theoretically, the low serum 1,25-(OH)$_2$D levels demonstrated by us in HIV-infected patients could have many explanations, including lack of precursor and binding protein. However, as 25D ordinarily is present in a 1000-fold higher concentration compared to 1,25-(OH)$_2$D, 25D would have to be virtually absent from the circulation to have any effect on the serum level of 1,25-
(OH)\textsubscript{2}D\textsubscript{3} and in the present study HIV-infected patients had 25D levels within the normal range. Likewise, slightly depressed, but still normal, levels of DBP make the lack of binding protein an unlikely explanation.

The rate of 1\textalpha-hydroxylation of 25D in the kidney normally determines the serum level of 1,25-(OH)\textsubscript{2}D, and the hydroxylation step is tightly regulated and influenced by a number of factors. Therefore, a reasonable assumption would be that inadequate 1\textalpha-hydroxylation might be a cause of 1,25-(OH)\textsubscript{2}D deficiency in HIV-infected patients. Normally, PTH as well as calcitonin stimulate 1,25-(OH)\textsubscript{2}D production, and low serum levels of these substances would decrease the rate of 1\textalpha-hydroxylation (3, 16) (Fig. 3). However, in HIV-infected patients, calcitonin levels are within the normal range, and although there was a slight reduction in PTH, serum levels of these parameters are not lower in patients with undetectable 1,25-(OH)\textsubscript{2}D than in other patients. This indicates that there may be some defect in PTH production/secretion in HIV-infected patients, but altered PTH production can not in itself explain the low 1,25-(OH)\textsubscript{2}D serum levels. HIV-infected patients with undetectable 1,25-(OH)\textsubscript{2}D concentrations had higher phosphate levels than the other patients, and high phosphate levels may inhibit 1\textalpha-hydroxylation of 25D. Yet, even if serum phosphate was significantly increased in patients with undetectable 1,25-(OH)\textsubscript{2}D levels, it was still within the normal range, so this cannot be the whole explanation for the decreased 1,25-(OH)\textsubscript{2}D levels. Although HIV-infected patients in the present study had serum creatinine levels within normal limits, a subclinical renal dysfunction affecting hydroxylation cannot be excluded.

One possible explanation for the lack of difference in PTH levels between patients with undetectable and normal serum levels of 1,25-(OH)\textsubscript{2}D in healthy controls and HIV-infected patients in CDC groups A, B, and C.
levels of 1,25-(OH)₂D may be that the stimulatory effect of PTH on 1α-hydroxylase activity is in some way inhibited in HIV-infected patients. Low 1,25-(OH)₂D levels would normally stimulate 1α-hydroxylase activity (3), and the lack of this feedback regulation further suggests an impairment of 1α-hydroxylase activity. The reason for this is unclear, but several not mutually exclusive factors may be involved. In vitro studies have demonstrated an effect of TNFα on 1α-hydroxylase activity in both renal and other tissues (17–19). Bikle et al. found that TNFα could both inhibit and enhance hydroxylase activity in keratinocytes, at least partly dependent on the degree of differentiation of these cells (18). Furthermore, TNFα has been found to induce vitamin D 1-hydroxylase activity in human macrophages (20, 21) and may be involved in the pathogenesis of hypercalcemia sometimes seen in human sarcoidosis and tuberculosis. However, during HIV infection there is a down-regulation of TNF receptors on monocytes/macrophages in advanced clinical disease (22), and granuloma formation is commonly not seen in mycobacterial infections in these patients. Thus, we suggest that this up-regulation of vitamin D 1-hydroxylase activity may not necessarily be operative in HIV-infected individuals. Of particular interest, TNFα appears to impair the stimulatory effect of PTH through mechanisms involving down-regulation of PTH receptors, impairment of protein kinase C activity, and inhibition of cAMP response after PTH stimulation (19, 21). Thus, although the exact role of enhanced TNFα activity in the induction of 1,25-(OH)₂D deficiency remains to be clarified, it may well contribute to 1,25-(OH)₂D deficiency in these patients at least partly by blocking the PTH stimulatory effect on vitamin D 1-hydroxylase. The strong correlation between elevated TNFα levels and decreased 1,25-(OH)₂D levels in HIV-infected patients is therefore interesting, and it is conceivable that this correlation may reflect an inhibitory effect on hydroxylation of 25D, possibly through blocking of the PTH effect.

In addition to a possible impairment of 1,25-(OH)₂D production, another explanation for low serum levels of 1,25-(OH)₂D may be increased degradation of 1,25-(OH)₂D. 1,25-(OH)₂D is important for cellular differentiation, especially for cells in the immune system (10, 23). HIV infection is characterized by an extremely high turnover of T lymphocytes (24). It is not inconceivable that increased need for 1,25-(OH)₂D for maturation of lymphocytes may account for at least part of the decreased serum levels of 1,25-(OH)₂D, or that there is a faster turnover of 1,25-(OH)₂D in immunologically activated, rapidly proliferating T cells.

Under normal conditions 1,25-(OH)₂D and PTH produce their net effects on calcium metabolism through complex interactions (3, 25), and 1,25-(OH)₂D deficiency is usually associated with secondary hyperparathyroidism, hypocalcemia, and subsequent decalcification of bone (4, 5, 26). Low levels of 1,25-(OH)₂D are traditionally believed to increase PTH production (27), and in vitro studies have shown that 1,25-(OH)₂D suppresses PTH production by directly suppressing PTH gene transcription (28). This pattern was not seen in HIV-infected patients, in whom the effect of 1,25-(OH)₂D deficiency on serum calcium was almost negligible. Furthermore, instead of hyperparathyroidism, these patients had slightly decreased PTH levels, in accordance with some other reports studying PTH metabolism in HIV infection (29, 30). However, recent studies have established that a calcium ion-sensing cell surface receptor may be the main regulator of PTH production (3, 31), and it is therefore possible that low 1,25-(OH)₂D levels do not stimulate PTH production as long as the serum calcium level remains normal.

The combination of markedly depressed 1,25-(OH)₂D levels and normal or only slightly decreased serum calcium may seem surprising. However, the independent role of 1,25-(OH)₂D in the regulation of serum calcium has recently been questioned (3, 32), and it is possible that the effect of 1,25-(OH)₂D deficiency on serum calcium levels is not exhibited until stressed by reduced calcium availability. Nevertheless, normal or only slightly decreased serum calcium in HIV-infected patients with persistently reduced 1,25-(OH)₂D levels indicate that compensatory mechanisms make calcium available for the extracellular compartment. Increased mobilization of calcium from bone may be one such mechanism, and indeed, increased bone resorption has been suggested in HIV-infected patients and other retroviral infections (33, 34). Again, this may be related to increased levels of TNFα and other proinflammatory cytokines (35).

In addition to its role in calcium homeostasis, 1,25-(OH)₂D influences the immune system both in vivo and in vitro. A substantial amount of experimental data from in vitro studies have established 1,25-(OH)₂D as a potent modulator of cells of the immune system (2, 10, 11, 36). In vivo, vitamin D deficiency has been associated with macrophage dysfunction and bacterial infections (8, 9, 36–39). Furthermore, until the advent of antibiotics, vitamin D was used to treat mycobacterial infections (6, 7), and infants with hereditary vitamin D-dependent rickets may die of serious pulmonary infections if the condition is not recognized and treated (5). Also, administration of 1,25-(OH)₂D to uremic patients has been shown to improve immune functions and modulate cytokine secretion (4, 40). Thus, even if the effect of severe 1,25-(OH)₂D deficiency on serum calcium is negligible in these patients, the correlation between low 1,25-(OH)₂D serum levels and clinically advanced HIV infection supports other in vivo observations that low 1,25-(OH)₂D serum levels may contribute to immunodeficiency.

Our observation of normocalcemic 1,25-(OH)₂D deficiency is not unique. Careful review of the literature reveals con-
TABLE 2. Serum levels of PTH, calcium (Ca), and phosphate (Pi) in clinical conditions characterized by severe deficiency of 1,25-(OH)2D and persistent immunoactivation

<table>
<thead>
<tr>
<th>Clinical condition</th>
<th>1,25-(OH)2D</th>
<th>PTH</th>
<th>Ca</th>
<th>Pi</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS (presented here)</td>
<td>↓</td>
<td>→</td>
<td>↓</td>
<td>→</td>
</tr>
<tr>
<td>Progressive mammary cancer with bone metastases (42)</td>
<td>↓</td>
<td>→</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>Tumour-induced rickets (41)</td>
<td>↓</td>
<td>?</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>Tuberculosis, myelomatosis (43)</td>
<td>↓</td>
<td>→</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>Septicemia with hypocalemia (44)</td>
<td>↑</td>
<td>→</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>Severe renal failure (4)</td>
<td>↑</td>
<td>→</td>
<td>→</td>
<td>→</td>
</tr>
</tbody>
</table>

Arrows indicate whether the serum level is moderately decreased (↓), severely decreased (→), normal (→), or moderately increased (↑). 1,25-(OH)2D-deficiency accompanying severe renal failure is included in the table for comparison. Reference numbers are in parentheses.

nitions with similar metabolic patterns (41–44) (Table 2). It is interesting that 1,25-(OH)2D deficiency with only minor alterations in serum PTH and calcium levels may be present in a number of conditions with marked immunological hyperactivity, such as tuberculosis, septicemia, and myeloma- tosis, as well as in AIDS. This is in sharp contrast to the pattern seen in severe renal failure (Table 2). In all reports concerning conditions with immunological hyperactivity and 1,25-(OH)2D deficiency, there seems to be an unknown factor contributing to inhibition of the 1α-hydroxylase activity. It is tempting to speculate that this factor may be related to activity of TNFα or other proinflammatory cyto-
kines, which are known to be increased in septicemia, HIV infection, and certain malignancies.

Interestingly, various types of antagonistic interaction between TNFα and 1,25-(OH)2D have been observed at the cellular level (45–47), and enhanced TNF levels may impair the action of 1,25-(OH)2D on various leukocytes. The low 1,25-(OH)2D and high TNFα levels observed here may therefore further impair the immune response in HIV-infected patients both independently and in combination and may represent an important feature of the pathogenesis of HIV-
related immunodeficiency. The combination of low 1,25-(OH)2D and high TNFα levels may also contribute to the impairment of immune function in other diseases characterized by markedly depressed 1,25-(OH)2D serum levels and immunological hyperactivity. Thus, the findings in the present study may not only represent a previously unrec-
ognized mechanism for induction of 1,25-(OH)2D deficiency in human disease, but may also reflect the importance of adequate serum levels of 1,25-(OH)2D for satisfactory performance of the immune system in man.

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