Serum Vitamin A Status in Acute Lymphocytic Leukemia of Childhood*

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ABSTRACT

Serum vitamin A status was assessed at the diagnosis in 31 children with common acute lymphocytic leukemia (CALL) and seven with T-cell leukemia (T-ALL). A control population consisting of 22 children was established to serve as one basis for comparison. Mean concentrations of vitamin A, retinol-binding protein (RBP) and prealbumin (PA) were significantly less (p < 0.01) in the sera of patients with T-ALL when compared with the controls. Similarly, mean RBP and PA levels were reduced (p < 0.01) in the sera of patients with CALL. In addition, “euretinolemic” and “hyporetinolemic” subgroups of CALL were identified comprising 74.2 percent and 25.8 percent of the patient population, respectively. The latter differed from the euretinolemic patients by being younger and showing an even greater decrease in the serum concentrations of RBP and PA. These findings indicate that there are factors affecting carrier proteins of vitamin A in childhood T-cell and common ALL; also, the data provide further evidence of the heterogeneity of common acute lymphocytic leukemia of childhood.

Introduction

Over the past several years, there has been an emergence of interest within the scientific and medical communities regarding the possible role of vitamins, especially E and A (retinol) and its precursor beta carotene, in both the risk of cancer and its chemoprevention.11,12,16,17,20,21,25,26,27 Recently, the use of the A vitamers, cis-retinoic acid and retinol ester, as cytodifferentiators and chemopreventive agents in certain types of nonlymphocytic leukemia has been attempted with some success.4,7,8,13 Moreover, there appears to be a possible role for such agents in the treatment of some patients with acute lymphocytic leukemia (ALL) of childhood given the observation that therapeutically achievable concentrations of retinoic acid can...
inhibit colony growth of bone marrow leukemic cells from children with common acute lymphocytic leukemia (CALL). However, before any clinical trials are undertaken to evaluate the efficacy of retinoids as adjunctive therapy in ALL patients, it would seem advisable to determine their underlying vitamin A status. In this study, the serum vitamin A status was examined in the two most frequently occurring subtypes of childhood ALL, namely common and T-cell ALL.

**Material and Methods**

**Study Population**

In accordance with the requirement of the Institutional Review Board of Cook-Fort Worth Children’s Medical Center, informed consent was obtained from the legal guardians of both patients and control subjects prior to their entry into this study. Thirty-eight children with ALL formed the patient population. Thirty-one of these fell into the immunophenotypic category of CALL as defined by the presence of CALL and Ia (HLA-DR) antigens on bone marrow lymphoblasts and negativity for pan T antigen. This group comprised 17 males and 14 females, ranging in age from one to 11 years. The remaining seven qualified as T-ALL as defined immunophenotypically by the presence of pan T antigen on bone marrow lymphoblasts. This group included 5 males and 2 females, ranging in age from two to 12 years. All were newly diagnosed, untreated cases. The controls consisted of 14 males and 8 females, ranging in age from one to 14 years. They were otherwise healthy children who were preparing to undergo elective otorhinolaryngologic procedures or herniorrhaphy.

Body weights on CALL and T-ALL patients and control subjects were included in the study. These were obtained at the time of diagnosis or upon admission for surgery and were converted to percentiles adjusted for age and sex according to data provided in the tables of Hamill and co-workers.

**Laboratory Analyses**

Patient preparation prior to obtaining the blood specimens in the CALL group included, in most cases, intravenous glucose (usually D5W • 1/4 NS) and transfusion with platelets and/or washed, packed red blood cells. The majority of the patients had also received allopurinol, but none had received antileukemic therapy. The controls had been fasting overnight in preparation for surgery and phlebotomies were performed shortly after induction of anesthesia with nitrous oxide, halothane and oxygen.

Hemograms were performed on patients and controls on the ELT-8/ds.*

Serum specimens from both groups were aliquoted, wrapped in aluminum foil to protect them from light, and frozen at −70°C until prepared for analysis. Vitamin A was quantified using a high performance liquid chromatographic technique according to a modification of the original procedure by Hansen and Warwick. Retinoids were assessed in representative cases by Ralph D. Ellefson, Ph.D. at Mayo Medical Laboratories, Rochester, MN using fractionation by high-pressure liquid chromatography and quantification by measurement of selective optical absorbance of the effluent from the chromatographic column.

Serum retinol-binding protein (RBP) and prealbumin (PA) levels were assayed by radial immunodiffusion using LC-Partigen and M-Partigen plates.†

The leukemic bone marrow cells in all cases were immunophenotyped at diag-

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**TABLE I**

Clinical Parameters and Serum Vitamin A Status* in T-cell (T-ALL) and Common Acute Lymphocytic Leukemia (CALL) of Childhood versus Controls

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Age (years)</th>
<th>Weight† (percentile)</th>
<th>Vitamin A (µmol/L)</th>
<th>RBP‡ (mg/dL)</th>
<th>PA§ (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I T-ALL (7)§</td>
<td>6.3 ± 1.6</td>
<td>57.9 ± 10.3</td>
<td>0.64 ± 0.08</td>
<td>2.1 ± 0.1</td>
<td>13.4 ± 0.8</td>
</tr>
<tr>
<td>II CALL (31)</td>
<td>4.5 ± 0.6</td>
<td>51.1 ± 6.1</td>
<td>1.05 ± 0.12</td>
<td>&lt; 2.1 ± 0.1</td>
<td>11.6 ± 0.6</td>
</tr>
<tr>
<td>III Fasting controls (22)</td>
<td>4.9 ± 0.8</td>
<td>58.9 ± 6.2</td>
<td>1.22 ± 0.08</td>
<td>3.0 ± 0.1</td>
<td>20.5 ± 0.6</td>
</tr>
</tbody>
</table>

P Values

| I versus II | NS† | NS | NS | NS | NS |
| I versus III | NS | NS | < 0.01 | < 0.01 | < 0.01 |
| II versus III | NS | NS | NS | < 0.01 | < 0.01 |

*All values expressed as mean ± standard error of the mean
†Weight in percentile adjusted for age and sex
‡RBP = Retinol-binding protein
§PA = Prealbumin
§Number in () = number of individual subjects in study
¶Two patients had concentrations below the lowest standard of the assay system and therefore, were reported as < 0.5 mg per dL
†NS = Not significant (i.e., P > 0.05)

**TABLE II**

Age and Serum Vitamin A Status* in Euretinolemic and Hyporetinolemic Subgroups of Common Acute Lymphocytic Leukemia (CALL) of Childhood

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Age (years)</th>
<th>Vitamin A (µmol/L)</th>
<th>RBP‡ (mg/dL)</th>
<th>PA§ (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I CALL patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A Euretinolemic (23)§</td>
<td>5.3 ± 0.7</td>
<td>1.26 ± 0.14</td>
<td>2.3 ± 0.1</td>
<td>12.7 ± 0.7</td>
</tr>
<tr>
<td>B Hyporetinolemic (8)</td>
<td>2.2 ± 0.4</td>
<td>0.45 ± 0.06</td>
<td>&lt; 1.3 ± 0.2¶</td>
<td>8.5 ± 0.8</td>
</tr>
<tr>
<td>II Age-matched controls (8)</td>
<td>2.4 ± 0.5</td>
<td>1.22 ± 0.13</td>
<td>3.0 ± 0.1</td>
<td>18.9 ± 0.6</td>
</tr>
</tbody>
</table>

P Values

| IA versus IB | < 0.05 | < 0.01 | < 0.01 | < 0.01 |
| IB versus II | NS¶ | < 0.01 | < 0.01 | < 0.01 |

*All values expressed as mean ± standard error of the mean
‡RBP = Retinol-binding protein
§PA = Prealbumin
¶Number in () = number of individual subjects in study
¶Two patients had concentrations below the lowest standard of the assay system and therefore, were reported as < 0.5 mg per dL
*NS = Not significant (i.e., P > 0.05)
(SEM) when applicable. Correlation coefficients as determined by linear regression analysis, Student’s t-test, and chi-square analysis with contingency table were performed using a microstat program on an IBM computer.

Results

Serum Vitamin A Concentrations in CALL

A lower mean vitamin A concentration was present in sera from the T-ALL group relative to the fasting controls (table I). In addition, eight of the 31 CALL patients (25.8 percent) but none (0/22) of the control subjects (p = 0.028) had serum vitamin A concentrations that fell into the deficient range, i.e., <20.0 µg per dl or <0.70 µmol per L, as established by the National Nutrition Survey. This coincides with published reference ranges for age in categorizing these CALL patients as “hyporetinolemic.” The remaining 23 CALL patients (74.2 percent) were classified as “euretinolemic.” Moreover, the hyporetinolemic subgroup was also distinguishable by the significantly younger mean age of its members (p < 0.05; table II) relative to the euretinolemic subgroup. However, the mean serum vitamin A concentration in the former remains significantly below (p < 0.01) that of age-matched controls (table II).
VITAMIN A STATUS IN ALL

0.25x + 10.689, R-squared: 0.076

Figure 3. Correlation between serum prealbumin (PA) concentrations and body weight percentiles adjusted for age and sex according to Hamill et al.⁹ in 31 patients with common acute lymphocytic leukemia (CALL) of childhood (r value = 0.28).

High-pressure liquid chromatography performed on representative cases from the T-ALL patients and the euretinol­emic and hyporetinolemic subgroups of CALL confirmed retinol as the only reti­noid, i.e. no retinyl esters, retinal, reti­noic acid or retinoate esters were detected.

SERUM RBP AND PA CONCENTRATIONS IN ALL

The mean concentrations of carrier proteins of retinol, RBP and PA, were significantly reduced in the sera of T-ALL and CALL patients as a whole as well as the hyporetinolemic subgroup of the latter (tables I and II). Additionally, there was a further decrease (p < 0.01) in the levels of each of these carrier pro­teins in the aforementioned hyporetino­lemic vis-à-vis the euretinolemic sub­group (table II). Overall, there was good correlation between RBP and PA con­centrations in CALL patients (r value = 0.84; figure 1) but not between RBP or PA concentrations and adjusted⁹ body weight percentiles in same (r values = 0.21 and 0.28, respectively; figures 2 and 3).

Discussion

The findings in this study indicate that the serum vitamin A concentration is sig­nificantly less in T-cell leukemia of childhood. Furthermore, our studies have identified a minor hyporetinolemic subgroup in common acute lymphocytic leukemia, and, thereby, further establish the heterogeneity of CALL.

Although the underlying basis for the reduced serum vitamin A concentration in T-ALL could be related in part to increased oxidative catabolism of the polyenic side-chain of retinol,³,²⁸ the concomitant decrease in the mean, serum levels of RBP and PA relative to control subjects provides a logical expla­nation for same. Similarly, the markedly decreased RBP and PA concentrations in the hyporetinolemic CALL patient relative to their euretinolemic counterparts suggest that deficiencies in these carrier proteins are playing a role in its decrease. That is to say, because retinol (vitamin A₁) is carried on RBP, which in turn is complexed with prealbumin,²⁸ a deficiency in one or both of these could account for decreased serum retinol con­centrations.

Although low serum RBP and PA con­centrations can reflect a state of general-
alized protein-calorie malnutrition, this does not appear to be present in either the T-ALL or the CALL group as evidenced by their relatively normal mean percentiles for body weight and the poor correlation in CALL patients between the latter and individual serum RBP and PA concentrations, respectively. Nevertheless, because of the good correlation between RBP and PA in CALL patients as a whole in this study (suggesting that both carrier proteins are being similarly affected by the disease process), a possible denominator has been sought. One established circumstance that might be contributing to the development of both is a simultaneous or pre-existent deficiency of zinc. Because zinc appears to be important in the synthesis of RBP and PA, the previous demonstration of reduced plasma zinc at diagnosis in acute lymphocytic leukemia of childhood by Delves and colleagues provides a possible explanation for our finding of decreased serum levels of RBP and PA in ALL.

Regardless of the mechanisms, however, decreased serum RBP and PA could have significant pathogenetic implications in patients with ALL. This should reduce both the delivery of retinol to all cells (including neoplastic lymphocytes) and, subsequently, the formation of retinoic acid from same. One consequence might be a loss of the cell modulating effects of retinol or its metabolite, retinoic acid, and, specifically, decreased regulation of ornithine decarboxylase, which is the first and probably rate limiting enzyme in polyamine synthesis. Furthermore, because the latter appears to be a sine qua non for both deoxyribonucleic acid (DNA) synthesis in mitogen-activated lymphocytes and the proliferation of leukemic lymphoblasts, decreased ornithine decarboxylase regulation could contribute, at least in part, to amplification of the leukemic process.

From a therapeutic standpoint, these carrier protein deficiencies theoretically could be corrected by supplemental zinc administration or circumvented by the administration of retinoic acid which is carried on albumin. Evaluation of the latter as an adjunctive therapy would, in our opinion, seem justifiable particularly in the hyporetinolemic subgroup of CALL and would be a logical extension of the in vitro studies of Findley and co-workers.

Acknowledgments

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References

8. Fontanna, J. A., Rogers, J. S., and Durham,


