Plasma Zinc Level and Thymic Hormone Activity in Young Cancer Patients

By Eugenio Mocchegiani, Paolo Paolucci, Donatella Granchi, Laura Cavallazzi, Lory Santarelli, and Nicola Fabris

It has been reported that in many neoplastic diseases, including leukemia, alterations in plasma zinc levels may frequently occur, although the causes for such alterations have yet to be clearly defined. Since zinc is required to induce biological activity to thymulin (Zn-FTS), a biochemical defined thymic hormone, and marginal zinc deficiencies may prevent its peripheral biological activation, we investigated the plasma level of zinc and of both active thymulin (Zn-FTS) and total zinc saturable thymulin (Zn-FTS + FTS) in 91 young patients affected by acute lymphoblastic leukemia (ALL) at various stages of the disease. It was discovered that the plasma zinc level was reduced at the onset and relapse, whereas in complete remission and in off-therapy it was in the normal range. Total zinc-saturable thymulin concentration did not change during the disease, whereas the active fraction was reduced at the onset and in relapse when compared with values observed in the other stages of the disease or in healthy controls. These data suggest that zinc plasma deficiency is present in ALL patients at the onset and during relapse, and that such a deficiency causes a decrease in the activity of thymulin despite a nearly normal production by the thymus. An impairment of peripheral immune efficiency in ALL patients is commonly found. The existence of positive correlations between zinc or active thymulin and peripheral immunological parameters (phystoagglutinin [PHA] and concanavalin A [ConA]) at various stages of the disease suggests a link between derangement of peripheral immune function, thymic hormone activity, and zinc failure. These findings, considered together, suggest the possibility of a carefully controlled clinical trial with zinc in ALL patients at the onset and in relapse even in the light of in vitro ineffectiveness of physiological zinc or thymulin concentrations on the duplicative index of human lymphoblastoid cells.

A NUMBER OF studies have documented the immunodeficiency state associated with the presence of neoplastic diseases, as well as the immunosuppressive effects of long-term chemotherapy, both of which are more evident in young cancer patients.

Cell-mediated immunity seems to be less effective than humoral immune response. This fact suggests that the maturation and differentiation of the T-cell lineage are primarily involved. Consequently, alterations of the functional efficiency of the thymus may represent one of the primary causes of such an immunodeficiency state, although histological evidence of thymic abnormality is not currently available.

More recent studies have been aimed at investigating, in young cancer patients, the plasma level of one of the best known thymic hormonal factors, ie, the factor thymique serique (FTS), more recently called thymulin in its zinc-bound biologically active form (Zn-FTS). These findings have shown a deep reduction in the plasma level of active thymulin, regardless of the stage of the neoplastic disease. Moreover, the same study documented in cancer patients the presence of plasma substances that inhibit the thymulin activity. Therefore, it is apparently not linked to zinc deficiency, which is a condition that can provoke the appearance of inactive Zn-unbound FTS molecules that inhibit the active form.

However, the normality of zinc turnover in cancer patients is an open question, since variations in both extracellular and intracellular zinc content have been reported in neoplastic diseases not dependent on the histological type or stage of disease, and the more or less balanced diet of patients. With regard to acute lymphoblastic leukemia (ALL), some studies have shown a significant reduction of plasma zinc values only at the onset of the disease, whereas other studies have failed to demonstrate any significant difference in respect to healthy control subjects. These findings, together with the known relevance of zinc, not only concerning thymic function, but also the efficiency of the entire immune system, have prompted us to measure the plasma levels of both active (Zn-FTS) and inactive (FTS) thymulin in patients with ALL at different stages of the disease, and to correlate them with the plasma zinc concentrations. An analysis of the effect of zinc and thymulin on leukemic cells has also been performed in view of possible clinical applications of zinc therapy.

MATERIALS AND METHODS

Patients. Ninety-one patients (45 males and 46 females; age, 18 months to 18 years) with ALL were studied. Twenty-seven were tested at the onset of the disease, 29 under maintenance therapy during complete remission, 7 during relapse, and 28 off-therapy for a span of 2 months to 2 years. Patients were treated according to chemotherapy and radiotherapy protocols recommended by the Italian Pediatric Oncologic Association (AIEPD). The data collected from the tests refer to different patients with the same disease and at the same treatment phase; therefore, they do not necessarily represent a longitudinal study of individual patients.

Complete remission is considered when less than 5% of the blast cells are found in the bone marrow and a total regression of all evident tumor is reached. A relapse is defined as the reappearance of more than 5% of the blast cells in the bone marrow and/or a development of a new malignancy site. Patients were defined as being off-
therapy when all therapy was finished, according to the individual protocol. All tests were performed with the written consent of the parents after the approval of the institutional human subjects’ review committee. ALL subjects were hospitalized patients of “Sant’Orsola” Hospital, III Pediatric Clinic, University of Bologna, Italy.

Plasma samples for both thymic hormone and zinc determination were separated from heparinized blood at 4°C and frozen at −70°C until used.

The analysis of peripheral immune function (T-cell subsets and mitogen responsiveness) was performed at random in ALL patients (5 subjects for each stage of the disease). The same patients were also assayed for thymulin (active and total) and zinc at various stages of the disease (Table 1). These patients came from the original selected population.

Normal subjects. Eighteen plasma samples from normal subjects, selected at random, from 2 years to 15 years old were used as controls. Normal subjects were chosen from patients admitted to the hospital for minor surgery.

Thymulin determination. Spleen cells from young mice include T and B lymphocytes carrying receptors for sheep erythrocytes (SRBCs) and, therefore, capable of forming rosettes when mixed with SRBCs. T and B rosettes can be distinguished by adding 10 μg/mL azathioprine, which selectively inhibits T-rosette formation. The azathioprine sensitivity of T cells is strictly dependent on the thymic endocrine function. Removal of the thymus in mice induces the complete disappearance of the azathioprine sensitivity, although it reappears when spleen cells from thymectomized mice are incubated with synthetic thymulin (Zn-FTS). This phenomenon represents the basis for the bioassay of thymulin in biological fluids. A bioassay is still required, since questions have been raised about the specificity of the radioimmunoassays (RIAs) developed until now.

Briefly, plasma samples were filtered through a Centrífoul Amicon membrane (Amicon, Lexington, MA) with a cutoff of 50,000 dal. Duplicate 50-μL aliquots of filtrate or serial dilutions of the filtrate made with Hanks’ solution were mixed with 200 μL of spleen cell suspension from thymectomized mice (final suspension, 7.5 × 10^6 cells/mL) and incubated at 37°C for 30 minutes.

After washing, the cells were resuspended in 250 μL of azathioprine solution (Welcome, London, UK) at a concentration of 10 μg/mL; this concentration inhibits the formation of rosettes by T lymphocytes, but not by non-T spleen cells. As a control, four aliquots of spleen cells were used. Azathioprine was added to two of them, while two contained just the medium. The cell suspension was then incubated at 37°C for 60 minutes, after which 250 μL of SRBCs suspension containing 12.5 × 10^9 cells/mL was added. After a further 5-minute incubation at 37°C, the cells were centrifuged at a cold temperature (4°C) at 100g for 5 minutes, resuspended for 5 minutes using a rotating mixer (10 cm in diameter, 8 rpm), and counted in a hemocytometer chamber. The rosette-forming cells (RFCs) present in 18,000 spleen cells were counted and values were recorded as RFCs per 1 × 10^6 cells. No inhibition of T rosetting was recorded in azathioprine-untreated tubes. Plasma filtrate tubes showed inhibition and the maximum dilution of plasma samples, inducing azathioprine sensitivity in 50% of RFCs from thymectomized mice, was taken as the thymulin titer; the percentage of RFCs that may become azathioprine-sensitive in the presence of excess thymulin concentration ranges between 50% and 65%.

This technique is specific for thymulin, since the assay is unaffected by other thymic hormones, and the rosette-inducing activity is removed completely by passing plasma samples through an antithymulin immunoadsorbent. The sensitivity of this bioassay allows for the detection of 1 pg/mL of synthetic thymulin (obtained from Serva, Heidelberg, Germany). Since in two consecutive blind assays no difference of more than one log₂ was found in all samples, the assay is considered reliable.

Determination of inactive plasma thymulin. The determination of inactive thymulin was based on the property of the inactive hormone to inhibit the biological effect of synthetic zinc-bound active thymulin (Serva). The method, originally developed by Bach and Beaumier to detect any plasma inhibitory activity on thymulin was later modified to induce specificity for the inactive thymulin, by concomitantly measuring thymulin activity and inhibitory effect after adding zinc sulfate to the plasma samples. If an inactive hormone is present (inhibitory effect), the addition of zinc ions induces the disappearance of the inhibitory effect and increases in the active thymulin titer. Briefly, 60 μL of plasma diluted 1:50 with Hanks’

### Table 1. Thymulin (active and inactive) Level, Zinc Concentration, Lymphocyte Subsets, and Mitogen Responsiveness in Young ALL Patients at Various Stages of Disease

<table>
<thead>
<tr>
<th>Immunological Parameters</th>
<th>At Diagnosis</th>
<th>Complete Remission</th>
<th>Relapse</th>
<th>Off-Therapy</th>
<th>Age-Matched Healthy Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age range (yr)</td>
<td>2-13</td>
<td>6-11</td>
<td>5-10</td>
<td>7-12</td>
<td>3-15</td>
</tr>
<tr>
<td>PHA response (cpm/culture × 10⁻³)</td>
<td>45.3 ± 5.9*</td>
<td>67.7 ± 8.6</td>
<td>30.7 ± 3.3*</td>
<td>86.7 ± 3.9</td>
<td>95.3 ± 7.5</td>
</tr>
<tr>
<td>ConA response (cpm/culture × 10⁻³)</td>
<td>60.9 ± 3.9*</td>
<td>81.5 ± 5.4</td>
<td>51.7 ± 4.7*</td>
<td>96.7 ± 5.3</td>
<td>110.8 ± 5.1</td>
</tr>
<tr>
<td>MNC (absolute no./μL)</td>
<td>2,815 ± 185t</td>
<td>2,560 ± 215t</td>
<td>ND</td>
<td>4,380 ± 170</td>
<td>4,665 ± 175</td>
</tr>
<tr>
<td>CD2 (absolute no./μL)</td>
<td>1,437 ± 77t</td>
<td>1,475 ± 87t</td>
<td>ND</td>
<td>2,830 ± 83</td>
<td>3,010 ± 81</td>
</tr>
<tr>
<td>CD3 (absolute no./μL)</td>
<td>1,319 ± 91t</td>
<td>1,420 ± 80t</td>
<td>ND</td>
<td>2,700 ± 73</td>
<td>2,625 ± 90</td>
</tr>
<tr>
<td>CD4 (absolute no./μL)</td>
<td>903 ± 15t</td>
<td>913 ± 20t</td>
<td>ND</td>
<td>1,965 ± 83</td>
<td>2,130 ± 83</td>
</tr>
<tr>
<td>CD8 (absolute no./μL)</td>
<td>987 ± 22</td>
<td>936 ± 36</td>
<td>ND</td>
<td>1,010 ± 22</td>
<td>1,075 ± 39</td>
</tr>
<tr>
<td>Active thymulin (log₂)</td>
<td>0.9 ± 0.6*</td>
<td>2.4 ± 0.55</td>
<td>1.7 ± 0.45*</td>
<td>4.1 ± 0.45</td>
<td>5.0 ± 0.62</td>
</tr>
<tr>
<td>Total thymulin (log₂)</td>
<td>4.2 ± 0.45</td>
<td>4.8 ± 0.84</td>
<td>4.4 ± 0.55</td>
<td>6.4 ± 0.65</td>
<td>5.5 ± 0.53</td>
</tr>
<tr>
<td>Inhibitory substances (pg/mL)</td>
<td>87.1 ± 2.70*</td>
<td>3.6 ± 1.19t</td>
<td>37.7 ± 9.70*</td>
<td>1.8 ± 0.90t</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td>Zinc (μg/dL)</td>
<td>59.3 ± 9.6*</td>
<td>90.8 ± 6.4</td>
<td>72.8 ± 5.9*</td>
<td>107.5 ± 6.2</td>
<td>112.2 ± 2.7</td>
</tr>
<tr>
<td>Range</td>
<td>(49-70)</td>
<td>(83-97)</td>
<td>(68-80)</td>
<td>(101-115)</td>
<td>(107-115)</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD. Number of patients studied: five, chosen at-random, for each group, belonging to the original selected population.

Abbreviation: ND, not determined.

* P < .01 when compared with values in complete remission, off-therapy, and healthy controls.

† P < .05 when compared with values of healthy controls.

‡ P < .01 when compared with values in off-treatment and healthy controls.
solution was mixed with 60 μL of different amounts of synthetic thymulin corresponding to a final concentration of 0.1, 0.5, 1.0, 10, 50, and 100 pg/mL. The 1.50 dilution of plasma was used to avoid interference by endogenous thymulin. The mixture was immediately filtered through a CF50 Amicon membrane (cone size, 50,000 d) for 30 minutes at 4°C. The filtrates were tested for thymulin activity in the rosette assay. The thymulin inhibitory activity was expressed as the highest concentration of synthetic thymulin inhibited by the plasma sample.

In the modified test, zinc sulfate was added to Hanks’ solution at a final concentration of 200 nmol/L. This zinc concentration was chosen on the basis of previous experiments performed with graded concentrations ranging from 1 pmol/L to 10 μmol/L, which showed that the 200-nmol/L concentration was optimal for unmasking inactive thymic hormone molecules.20

Zinc determination. Plasma zinc was measured using the method reported by Fernandez and Kahn.21 Blood samples were collected into fluorinated tubes to avoid contamination (no. 115317, L.P., Milan, Italy) and centrifuged for 20 minutes, then at 300 g for 10 minutes. Plasma samples were then frozen at −70°C and stored until use. Zinc was determined by atomic absorption spectrophotometry against a standard reference as suggested by Evenson and Warren.22

Lymphocyte subsets’ analysis. Mononuclear cells (MNCs) from 20 ALL patients at various stages of disease were obtained from peripheral blood by Ficoll-Hypaque (Pharmacia, Piscataway, NJ) density gradient sedimentation. The MNCs were washed and resuspended in phosphate-buffered saline (PBS) containing 5% heat-inactivated fetal calf serum (FCS) in the amount of 1 × 10^6/mL. Fifty microliters of MNCs, isolated as described above, were incubated at 4°C for 30 minutes with 50 μL of monoclonal antibodies for CD antigens, and conjugated with goat antimouse fluorescein isothiocyanate (FITC) at a final dilution of 1:100. After washing with PBSFCS, the cells were analysed in a fluorescence-activated cell sorter (Epics V; Coulter, Hialeah, FL). The positively stained cells were those with a greater fluorescence intensity than cells labeled with the second antibody alone (FITC) and were expressed as absolute number per microliter. Approximately 10^5 randomly accumulated viable cells were analyzed. The monoclonal antibodies used were CD2 (E-rosette T cells), CD3 (total T cells), CD4 (T-helper cells) and CD8 (T suppressor/cytotoxic cells; Becton Dickinson, San Jose, CA).

Lymphocyte proliferative response. The lymphocyte proliferative response was assessed in 20 young ALL patients at various stages of the disease (5 at onset, five in complete remission, five at relapse, and five off-therapy). Ficoll-Hypaque–enriched peripheral blood MNCs, at a concentration of 1 × 10^6/mL in RPMI 1640 (GIBCO, Gaithersburg, MD) containing 10% FCS (GIBCO), penicillin (100 U/mL), and streptomycin (100 μg/mL), were used. Aliquots of 0.1 mL were distributed in microwells (Nunc, Roskilde, Denmark). Phytohemagglutinin (PHA) (DIFCO, Detroit, MI) to reach final concentrations of 0.0125 μg/mL and of 0.05 μg/mL and concanavalin A (ConA) (Serva, Heidelberg, Germany) to reach final concentrations of 1 μg/mL and 0.5 μg/mL were added in the amount of 10 μL/well. After 48 hours of incubation in a 5% CO2–air environment at 37°C, H2-Td (Amersham, Littlechalfont, UK; specific activity, 2 Ci/mL) was added in the amount of 1 μCi/well. After additional 18 hours of incubation in a CO2 atmosphere, cultures were killed by means of a cell harvester (Skatron, Lier, Norway) and radioactivity was measured by a scintillation counter (Packard, Milan, Italy). All cultures were performed in quadruplicate, and the results, referring to the highest responses regardless of the mitogen concentration used, are expressed in cpm per culture. Lymphoblastoid cells’ proliferative response. To evaluate the effect of zinc ions or thymulin on leukemic cells, tests were performed using lymphoblastoid cell cultures established from the bone marrow of five young ALL patients (three with ALL type T and two with ALL common type) at the onset of the disease and by some lymphoblastoid cell lines, such as HL-60, KG-1, JM, MolM-4, and Daudi.

Bone marrow blast cells were obtained by iliac crest aspirations. Lymphoblastoid cells were suspended at a concentration of 1 × 10^3/mL in RPMI-1640 containing 10% heat-inactivated FCS, penicillin (100 U/mL), and streptomycin (100 μg/mL). Their vitality was determined by trypan blue exclusion. Aliquots of 0.1 mL were distributed in microwells.

Zinc sulfate was added in the amount of 10 μL/well to reach a final concentration of zinc ions of 10^−4, 10^−5, and 10^−6 mol/L.23 Synthetic active thymulin (Zn-FTS) (Serva) was added in the amount of 10 μL/well to reach a final concentration of 10^−3, 10^−4, and 10^−5 mol/L H3-Td was added in the amount of 1 μCi/well.

After 20 hours of incubation in a 5% CO2–air environment at 37°C, all cultures were killed by means of a cell harvester, and radioactivity was measured by a scintillation counter. All cultures were performed in quadruplicate and the results are expressed in cpm per culture.

Statistical analysis. Differences between the groups and controls were assessed by Student’s t-test. The correlation was determined by linear regression analysis and the least-squares methods. Differences were considered significant at P < .05. Variations during the various stages of the disease were calculated using analysis of variance (ANOVA). Furthermore, ANOVA (one-way and two-way) was used for variations from in vitro data of blast cell cultures.

RESULTS

Zinc-dependency of low thymulin levels in ALL patients. Figure 1 shows that ALL patients at the onset of the disease and during relapse have low plasma levels of active thymulin (Zn-FTS) when compared with values of age-matched control subjects (P < .01), whereas the concentrations of inhibitory substances (inactive thymulin) are extremely high (P < .01). In the in vitro addition of zinc sulfate to the ALL plasma samples at the onset and during relapse induces a strong reduction of inhibitory activities and the reappearance of thymulin activity (total thymulin = Zn-FTS + FTS) to levels comparable to those present in plasma samples from healthy control subjects. By contrast, the plasma active thymulin levels show, in complete remission and in off-therapy stages, only slight and not significantly reduced values when compared with those observed in young healthy controls. However, a small but statistically significant increase of the plasma concentration of inhibitory substances is present (P < .05), and the in vitro zinc addition induces a nearly complete disappearance of the inhibitory activity.

A statistically significant reduction in plasma zinc levels is present at the onset and during relapse of the disease when compared with the values of healthy controls (P < .01) (Fig 1), whereas no significant differences are observed in complete remission and in off-therapy stages. If the plasma zinc levels, regardless of stage of disease, are plotted against the corresponding values of in vitro thymic hormone saturable fraction (Zn-FTS + FTS/Zn-FTS), a significant positive correlation is present (r = .87; P < .01) (Fig 2).
Lymphocyte subsets and mitogen responsiveness in ALL patients. Table 1 shows that both at the time of diagnosis and in complete remission, ALL patients show reduced MNC counts, as well as reduced absolute numbers of CD2+, CD3+, and CD4+ cells, when compared with the values of age-matched healthy controls (P < .01). However, off-therapy patients with ALL showed normal counts of MNCs and lymphocyte subsets (CD2+, CD3+, and CD4+). CD8+ cells did not indicate significant differences among the patient groups and the controls.

The proliferative responses of peripheral blood lymphocytes to PHA and ConA are significantly reduced (P < .01) in ALL patients at the time of diagnosis and during relapse of the disease as compared with the values observed in the other stages of the disease or in normal controls.

Table 1 also shows the plasma thymulin (active and total) levels, plasma inhibitory substance (inactive thymulin) levels, and plasma zinc concentrations at various stages of the disease in the same ALL patients who were tested for the peripheral immune efficiency. With regard to thymulin levels and zinc concentrations, the data reported in Table 1 show a strong reduction of both active thymulin and zinc plasma levels, whereas the concentration of inactive thymulin is extremely high at the onset and during relapse of the disease when compared with controls (P < .01). However, in complete remission and in off-therapy patients, small but significant variations of inactive thymulin are present when compared with the values of age-matched healthy subjects (P < .05). Nevertheless, when the values of PHA and ConA mitogen responsiveness were plotted against plasma zinc concentrations or active thymulin plasma levels at various stages of the disease, significant positive correlations were found (Fig 3A, C, D, and F) and negative ones against inhibitory substances (inactive thymulin) (Fig 3B and E).

Effect of in vitro addition of zinc and active thymulin (Zn-FTS) on duplicative index of lymphoblastoid cells. The possible effect of zinc or of active thymulin (Zn-FTS) on the proliferative activity of lymphoblastoid cells was studied on cell lines established from bone marrow of ALL patients and on laboratory lymphoblastoid cell lines. The phenotypic characteristics of ALL patients, from which cell cultures were derived, are reported in Table 2.

Table 3 shows that zinc does not increase the proliferative rate of human lymphoblastoid cells at 10^-6 or 10^-5 mol/L concentrations when compared with values of control cultures, while a modest and statistically significant increment is observed at the concentration of 10^-4 mol/L (P < .05). On the contrary, the proliferation rate of human blast cell cultures is significantly reduced when active thymulin is added at the concentration of 10^-5 mol/L (P < .05), whereas at lower thymulin concentrations, slight or no variation is observed. Such a decrement of proliferative rate is observable both in ALL-T and ALL-C patients. The effect of zinc and active thymulin on the proliferative rate of laboratory lymphoblastoid cell lines is also reported in Table 3. The in vitro addition of active thymulin is also able to induce a slight but significant decrement of the proliferative rate in these cells (P < .05) when compared with untreated control cultures. Zinc, by contrast, does not induce a significant increment of the proliferative rate. Only at the concentration of 10^-4 mol/L in the JM cell line is zinc able to induce a significant increment of the duplicative index (P < .05). Furthermore, it is relevant to note that, although a reduction is present in the proliferative rate when zinc, at the dose of 10^-3 mol/L, is added in vitro to blast cell cultures, a statistically significant variation of the duplicative index is observed only for active thymulin (10^-3 mol/L) (P < .05).

DISCUSSION

The present report shows that both plasma zinc level and thymulin activity are strongly reduced in leukemic patients at the onset and during relapse of the disease when compared with values of age-matched controls, whereas concomitantly high levels of inhibitory substances on thymic peptide activity are observed. At other stages of the disease, plasma concentrations of thymulin, inhibitory substances, and zinc are close to those observed in age-matched young healthy subjects. The findings on plasma zinc levels are in accordance with previous studies. They show a reduction of plasma zinc level at the onset of the disease in young ALL patients and a recovery of zinc values during complete remission and in off-therapy patients, whereas they are in disagreement with previous reports that show normal plasma zinc values at the onset of the disease. However, it is interesting to note that in these last studies the range of zinc values in healthy subjects is extremely wide (from 60 to
300 μg/dL and the number of patients is quite limited, whereas in our measurements, as reported by other investigators, the zinc range is much more restricted and the number of patients is large.

The causes for the reduction of zinc plasma levels in ALL patients are not yet established. It has been suggested recently that the low zinc values observed in many neoplastic diseases, including leukemia, may result from an inadequate intake of zinc due to malnutrition or, especially during the relapse stage of the disease, it may be a consequence of the administration of antimetabolite drugs, which have been shown to enhance urinary secretion of bivalent cations. Although a definite interpretation of the low zinc levels in ALL patients is still not available, these findings suggest the presence of a low bioavailability of zinc ions at the onset and relapse of the disease.

The relevance of zinc in maintaining immune system efficiency is, at present, well documented. In particular, zinc plays a key role in inducing biological activity to one of the best known thymic hormones, the facteur timique sérique (FTS), more recently called thymulin in its zinc-bound active form.

Thymulin is a zinc conjugate of a nonapeptide FTS synthesized by the epithelial cells of the thymus; its biological activity resides in the conjugate form, whereas the zinc-unbound form is inactive and can inhibit the active form. The mechanism of such an inhibition is unknown; it can be suggested that the inactive zinc-unbound form may compete with the biologically active form (thymulin) for FTS receptors, as do synthetic FTS analogs.

It has been shown that in marginal zinc deficiencies, dependent on mild reduction of zinc intake or associated with sickle cell anemia, acrodermatite enteropathica, Down's syndrome, and Duchenne's syndrome, acquired immunodeficiency syndrome, and juvenile type 1 diabetes, the thymic peptide FTS may be synthesized and secreted in normal amounts, but only a fraction of it becomes bound to zinc ions and is therefore active. The inactive zinc-unbound form present in these conditions may be revealed by the fact that it exerts an inhibitory action on the active form and that in vitro addition of zinc ions causes the disappearance of this inhibitory activity with a concomitant increase of the active form.

Our findings clearly show that in the leukemic condition, at the onset and during relapse of the disease, low thymulin levels are not due to a failure of the endocrine thymus but to a reduced peripheral saturation of the thymic peptide by zinc ions. Such an assumption is supported by the fact that in vitro addition of zinc to the ALL plasma samples at the onset and during relapse was able to induce a complete saturation of thymic hormone molecules present, whereas in complete remission and in off-therapy patients, only a slight effect of in vitro addition of zinc to plasma samples on thymulin activity was observed. This interpretation is further supported by the strict correlation existing between plasma zinc level and the degree of in vitro zinc saturation of thymic hormone regardless of the stage of the disease.

A major criticism of this interpretation is whether a 20% to 30% reduction of plasma level of zinc may be of practical significance. However, note that thymulin (kd, 10⁻⁷ mol/L) is not first in the affinity scale of available zinc; being preceded by nerve growth factor (kd, 10⁻¹¹ mol/L) and metallothioneins (kd, 10⁻⁸ mol/L), it is nearly equal to metalloproteins (kd, 10⁻⁷ mol/L) and followed by many other zinc-
dependent enzymes such as alkaline phosphatase (kd, 10^{-6} \text{ mol/L}).^{36-40} \text{ Note that the much lower plasma concentrations of thymulin (3 mg/dL) in respect to the concentrations of other zinc-bound proteins, even with lower kd, can further reduce zinc availability for the hormone. Zinc supplementation experiments may confirm the role played by the 20% to 30% to decrease in plasma zinc observed in ALL patients at the onset and during relapse of the disease. In other human marginal zinc deficiencies, such as in Down's syndrome and uremic conditions, both of which are characterized by a \leq 20% reduction of plasma zinc level and low thymulin titers, zinc supplementation had a beneficial effect in recovering plasma zinc levels, thymulin titers, and some crippled immune functions.}^{41,42}

However, it must be noted that the in vitro addition of zinc to plasma of ALL patients does not totally eliminate the presence of inhibitory substances, as inhibiting activity remains, particularly at the onset and during relapse. Such a finding is difficult to explain. The existence of low–molecular weight substances, other than zinc-unbound FTS, capable of inhibiting active thymulin, may be suggested. Some experimental findings support this, but their nature is, at present, unknown.^{43}

Profound disturbance of the immune system is frequently found in patients suffering from neoplastic diseases, including leukemia, either at diagnosis or after treatment with radiation or chemotherapy.^{44-48}

Cell-mediated immunity, assessed by the measurement of both the absolute number of T-cell subsets and mitogen responsiveness, is impaired in leukemic patients even after
therapy has been discontinued.\textsuperscript{49,50} Our data are in agreement with these findings. A significant decrement both in T-cell subsets and in mitogen responsiveness was present at the onset and during relapse of the disease in our ALL patients. These parameters are normal in the off-therapy stage, but are in disagreement with other reports.\textsuperscript{6} The existence of positive correlations among PHA and ConA mitogen responsiveness and active thymulin or zinc during the various stages of the disease clearly suggest a causative relationship between derangement of peripheral immune function at the onset and relapse of the disease and thymic hormone or zinc failure.

A clinical trial with zinc in these patients at the onset and during relapse of the disease might, therefore, have a beneficial effect in order to increase the biological activity of the thymic hormones, as well as to recover the crippled immune functions, as demonstrated in other human marginal zinc deficiencies,\textsuperscript{41,42} in some neoplastic diseases,\textsuperscript{31-35} and in physiological aging,\textsuperscript{56} all of which are characterized by reduction of zinc plasma levels and by low thymulin titers.

However, the recent discovery that zinc induces mitogenesis,\textsuperscript{57} raises the question of its possible effect on neoplastic cells. An analysis of the effect of zinc on lymphoblastoid cells has shown that zinc is mitogenic only when used at pharmacologic doses, whereas at physiologic doses it has no effect on neoplastic cellular duplication.

By contrast, thymulin in its zinc-bound active form (Zn-FTS), at the dose of $10^{-3}$ mol/L corresponding to the physiologic amount of thymulin in the blood circulation of young healthy individuals,\textsuperscript{3} induced a slight but significant decrement of the duplicative index both in human lymphoblastoid cells and in some laboratory blast cell lines. The mechanism by which active thymulin affects the duplicative rate of blast cells remains unknown.

According to these last findings, the proposed clinical trial with physiologic doses of zinc, in particular at the onset and during relapse of the disease, in order to induce a complete saturation of thymic hormone and possibly an improvement of peripheral immune efficiency, may find a further rationale.

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REFERENCES

37. Dunn MF, Pattison SE, Storm MC, Quiel E: Comparison of the zinc binding site in the 7s nerve growth factor and the zinc-insulin hexamer. Biochemistry 19:718, 1980
39. Baudier D, Gerard D: Ions binding to S100 protein: Structural changes induced by calcium and zinc on S100 and S100b proteins. Biochemistry 22:3360, 1983
Plasma zinc level and thymic hormone activity in young cancer patients

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