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Effects of Soluble TNF Receptor II (sTNF-RII), IL-1 Receptor Antagonist (IL-1ra), Tumor Load and Hypermetabolism on Malnutrition in Children with Acute Leukemia

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Abstract
Objective: Soluble tumor necrosis factor receptor II (sTNF-RII) and interleukin-1 receptor antagonist (IL-1ra) might modulate nutritional status in acute leukemia since they are inhibitors of tumor necrosis factor-α and interleukin-1 that can induce tissue wasting. On the other hand, tumor load and hypermetabolism may induce malnutrition. We determined whether serum levels of sTNF-RII and IL-1ra are upregulated to prevent overt malnutrition and whether tumor load and hypermetabolism induce overt malnutrition.
Methods: We examined 31 children with newly diagnosed acute leukemia and correlated sTNF-RII, IL-1ra, tumor load and energy expenditure to anthropometric characteristics (weight, weight for height, height, body mass index, fat free mass) and serum protein concentrations (albumin, transferrin, prealbumin). As controls, 68 healthy children were examined for anthropometric characteristics; 33 healthy controls were included for cytokine analysis and biochemical indices.
Results: We found no correlations between sTNF-RII, IL-1ra, tumor load and energy expenditure and anthropometric characteristics or protein concentrations. Mean sTNF-RII level was significantly, mean IL-1ra level slightly increased (223% and 113% of the controls). 29% of the children had a high tumor load (> 100.000/µl white blood cells) and 53% had hypermetabolism (resting energy expenditure > 110% of predicted). Anthropometric characteristics were similar to those in controls, however, serum protein concentrations were decreased.
Conclusion: sTNF-RII and IL-1ra are upregulated in children with leukemia and may therefore prevent overt malnutrition. Tumor load and hypermetabolism do not induce overt malnutrition. The children presented with an early stage of malnutrition as evidenced by low serum protein concentrations but normal anthropometric characteristics.

Key words: nutritional status; childhood leukemia; tumor load; energy expenditure; soluble tumor necrosis factor receptor II; interleukin-1 receptor antagonist

Introduction

Previous investigations have demonstrated that malnutrition occurs between 0% and 15% in children with newly diagnosed acute leukemia. To evaluate malnutrition, weight, height or body mass index [1], arm anthropometry or bioelectrical impedance analysis [2-6] or serum protein concentrations [3, 7] were used. However, till now, factors that prevent or induce malnutrition in acute leukemia are not well known. Virtually no information exists regarding the influence of soluble tumor necrosis factor receptor II (sTNF-RII) and interleukin-1 receptor antagonist (IL-1ra). They are natural inhibitors of tumor necrosis factor-α (TNF-α) [8] and interleukin-1 (IL-1) [9] that can induce tissue wasting [10]. In addition, not much is known whether tumor load and hypermetabolism induce malnutrition in patients with leukemia. In 15 children studied, resting energy expenditure and anthropometric characteristics were not different between leukemic children and controls [4]. However, these 15 children had only low-risk acute lymphoblastic leukemia. Stallings et al. [11] showed that the three of nine examined children with a high white blood cell count were hypermetabolic (ratio of measured to predicted resting energy expenditure >110%). However, they did not correlate tumor load and hypermetabolism to nutritional parameters.

Therefore, we asked 1) whether serum levels of sTNF-RII and IL-1ra are upregulated and may prevent overt malnutrition and 2) whether tumor load and severity of hypermetabolism induce overt malnutrition in childhood acute leukemia. To answer these questions, we studied 31 children with newly diagnosed acute leukemia and correlated sTNF-RII, IL-1ra, tumor load and energy expenditure to anthropometric characteristics (weight, weight for height, height, body mass index, fat free mass) and serum protein concentrations (albumin, transferrin, prealbumin).

Patients and Methods

Patients

Thirty one children with acute leukemia (29 with acute lymphoblastic leukemia [ALL], 3 with acute myelo-

blastic leukemia [AML]) were investigated at first diagnosis of their disease and before initiation of hydration and antineoplastic treatment (mean age ± SD: 7.3 ± 5.0 years, range: 0.23-15.5 years, 15 female). Exclusion criterion was parental refusal to participate in the study. Children with AML were included since both entities, ALL and AML, develop as a consequence of malignant transformation of a single hematopoetic progenitor cell resulting in rapid proliferation of blasts and therefore in rapid diagnosis. In all 31 children anthropometric characteristics were studied. Resting energy expenditure (metabolism) was measured in 15 of the 31 children, while the other children refused the procedure. sTNF-RII, IL1-ra and plasma protein concentrations could not be measured in all children because of limited volumes of blood samples available. To determine that plasma proteins were not altered due to other conditions, infections were excluded by the absence of fever > 38.5 °C and/or positive blood or urine cultures, changes in hydration by the presence or absence of peripheral edema and liver diseases by normal liver function tests.

Controls

As controls for anthropometric characteristics, 68 healthy children (mean age ± SD: 8.2 ± 5.2 years, range: 0.3-18.4 years, 28 female) were examined. In these healthy children, blood sampling was not possible for ethical reasons. Therefore, as controls for cytokine antagonist levels and biochemical indices, we recruited 33 children attending our medical out-patient department for diagnostic procedures without significant acute or chronic illness (mean age ± SD: 9.4 ± 4.7 years, range: 0.8-16.4 years, 22 female). These controls showed no significant differences in age, sex and anthropometry from the leukemic children. The leukemia and control groups were evaluated at the same time. Informed consent was obtained from the parents of all children studied.

Methods

Serum levels of sTNF-RII and IL-1ra were measured using an enzyme-linked immunosorbent assay method (Quantikine™ human sTNF RII and Quantikine™ human IL-1ra, R&D Systems, DPC Biermann, Bad Nauheim, Germany) according to the manufacturer`s instructions. All determinations were done in duplicate. Fresh blood samples of patients and controls were immediately centrifuged and serum samples were stored at –80 °C until use.

To evaluate tumor load, the initial white blood cell (WBC) count was measured. Children with a WBC count greater than 100.000/mm^3 were considered to have a high tumor load.

Resting energy expenditure is defined as the minimum energy expenditure for maintaining essential body functions under standardised resting conditions, 12 hours postprandial in a neutral thermal environment. In this study, resting energy expenditure was determined by indirect calorimetry using an open-circuit ventilated-hood system (Deltatrac II; DATEX, Helsinki). The measurements were conducted for a minimum of 30 minutes after an overnight fast when stable energy expenditure was achieved. Urine was collected for 24 hours for determination of urinary nitrogen excretion. Resting energy expenditure was calculated from V_{O2} consumption, V_{CO2} production and urinary nitrogen excretion by using the formula: $REE = 5.50*V_{O2} + 1.76 * V_{CO2} - 1.99*U_N$. The predicted resting energy expenditure was calculated by formulas according to Fleish [12] and for infants according to Schofield [13]. Hypermetabolism was defined as the percentage of the measured to the predicted resting energy expenditure exceeding 110%. This level was used because it has been demonstrated that 95% of normal individuals have a measured resting energy expenditure within 10% of that predicted by the Harris-Benedict formulae, that means within a range of 90% and 110% [14].

All patients and controls underwent detailed anthropometry. Weight was measured to the nearest of 0,1 kg using an electronic scale (SECA, Vogel & Halke, Hamburg, Germany) and height (or supine length for children < 2 years) to the nearest of 0,1 cm using a stadiometer (System Dr. Keller I, Limbach-O, Germany). Weight, height and weight for height are expressed as mean percentages of the median reference values of age and sex specific growth charts for a control European population [15]. For body mass index (= weight/height squared), reference values were taken from the NHANES I and NHANES II surveys compiled by Frisancho [16]. Fat free mass was determined by bioelectrical impedance analysis (BIA 2000-M, Data-Input, Frankfurt, Germany) and was calculated by using the equation of Houtkooper et al. [17]. Fat free mass is expressed in kg body weight.

For the assessment of plasma protein concentrations, albumin, prealbumin and transferrin were determined by standard methods on automated clinical chemistry analyzers. In brief, albumin was determined by the bromocresolgreen method on a Hitachi 917, prealbumin and transferrin by immunoturbidimetry on an Intergra 700 (both Roche Diagnostics, Stuttgart, Germany) with reagents from the same supplier. The following plasma protein concentrations were considered abnormal [3, 16]: albumin < 3.5 g/dl, prealbumin < 16 mg/dl and transferrin < 200 mg/dl. Albumin was measured in 23, transferrin in 19 and prealbumin in 18 of the 31 children.

Statistical Analysis

Data are expressed as mean ± SD. Statistical significance was examined by the Mann-Whitney-U test. The Spearman correlation coefficient was used for correlation analyses. Differences were considered significant at $P < 0.05$. The SPSS software (SPSS Inc., Chicago, Il, USA) was used for statistical analyses.

Results

The serum levels of both cytokine antagonists were upregulated (Table 1). In 22 leukemic children, the mean sTNF-RII level was 223% of the level in 25 control subjects ($P<0.001$). The mean level of IL-1ra was 113% of the level in 31 controls. There were no corre-

Table 1. Serum levels of sTNF-RII and IL-1ra in 31 children with acute leukemia and 33 controls. Data are expressed as means ± SD. The mean levels of sTNF-RII and IL-1ra were higher in the leukemia group than in the controls (* = P <0.001).

	Leukemia group			**Control goup**		
	No	mean±SD	(range)	No	mean ±SD	range
Age	31	7.3 ± 5.0 y	(0.2-15,5)	33	9.4 ± 4.7 y	(0.8-16.4)
sTNF-RII	22	5534 ± 2887 pg/ml*	(1132-11606)	25	1713 ± 713 pg/ml*	(694-3308)
IL-1ra	22	641 ± 1773 pg/ml	(4-8488)	31	300 ± 250 pg/ml	(0.5-769)

Table 2. Anthropometric characteristics of 31 children with acute leukemia and 68 healthy controls. Mean ± SD is given. Weight, weight-for-height, height and body mass index are expressed as percentages of median reference values. Fat free mass is expressed in kg body weight and was examined by bioelectrical impedance analysis. No significant differences were found for any of these parameters.

	Leukemia children	**Healthy controls**
Number	31	68
Sex (f/m)	15/16	28/40
Age (years)	7.3 ± 5.0	8.2 ± 5.2
Weight%	109 ± 22	106 ± 22
Weight-for-height%	106 ± 13	104 ± 15
Height%	102 ± 4	101 ± 6
Body mass index%	102 ± 14	100 ± 14
Fat free mass (kg)	23.3 ± 13.8	24.6 ± 14.3

lations between both cytokine antagonists and anthropometric characteristics or plasma protein concentrations. However, tumor load was significantly correlated to sTNF-RII (r = 0.44, P = 0.039) but not to IL-1ra. The 6 children with >100.000/µl WBCs had a significantly higher mean level of sTNF-RII than the 16 patients with a lower tumor load (7844 ± 3027 (SD) vs. 4668 ± 2384 pg/ml, P = 0.013). In contrast, these 6 children with >100.000/µl WBCs had an even lower mean level of IL-1ra than the 16 children with a lower tumor load (194 ± 105 vs. 809 ± 2070 pg/ml).

Hypermetabolism was present in 53% (8/15) of leukemic patients. Mean resting energy expenditure, expressed as percentage predicted, was 113 ± 17% (range: 88-148%). However, although half of all leukemic children were hypermetabolic, metabolism did not influence anthropometric characteristics and plasma protein concentrations. Again, tumor load correlated with hypermetabolism. There was a trend to a higher mean level of measured to predicted resting energy expenditure in the 3 children with a high tumor load than in the 12 children with a low tumor load (119 ± 4% [range: 115-121%] vs. 111 ± 19% [range: 88-148%]). All 3 children with a high tumor load were hypermetabolic, although not different in nutritional indices from controls, whereas only 5 of 12 children (42%) with a low tumor load were hypermetabolic.

Mean weight, height, weight for height, body mass index and fat free mass were not different between the leukemic patients and the control group (Table 2). The 9 patients with a high tumor load had no different anthropometric characteristics than the 22 children with lower leukemia load and the healthy control group. Thus, children with acute leukemia independant of tumor load do not present with overt malnutrition at diagnosis of their disease.

Mean levels of plasma protein concentrations (albumin, transferrin and prealbumin) were significantly lower in the leukemia than in the control group (P < 0.001) (Table 3). The 9 children with a high tumor load had similar mean plasma protein concentrations

Table 3. Plasma protein concentrations in children with acute leukemia compared to healthy controls. Mean ± SD is given.The number of children with abnormal plasma protein concentrations was significantly higher in the leukemia than in the control group (+ = P< 0.001). In addition, the mean level was significantly lower in the leukemia than in the control group (* = P <0.001).

		Leukemia group				**Control goup**			
	abnormal low	No	No low levels	mean±SD	(range)	No	No low levels	mean±SD	(range)
Age		31		7.3 ± 5.0	(0.2-15.5)	33		9.4 ± 4.7	(0.8-16.4)
Albumin	<3.5 mg/dl	23	9 (39%)+	3.6 ± 0.5*	(2.9-4.7)	30	0 (0%)+	4.4 ± 0,3*	(4.0-4.9)
Transferrin	<200 mg/dl	19	9 (47%)+	212 ± 46*	(128-284)	26	0 (0%)+	280 ± 35*	(203-335)
Prealbumin	<16 mg/dl	18	12 (67%)+	14.0 ± 3.9*	(8.8-23.8)	32	3 (9%)+	22.4 ± 4.6*	(10.8-31.6)

than the other 22 children with a low tumor load, but significantly lower concentrations than controls ($P \leq 0.01$). These decreased plasma protein concentrations were not related to acute phase reactions: 5 of the 31 children had fever > 38.5 °C, but none had positive blood or urine cultures. The plasma protein concentrations were also significantly decreased when the 5 children with fever were excluded from the analysis. In addition, no child had peripheral edema or liver failure as evidenced by normal liver functions tests (data not shown).

Discussion

This study shows that children with acute leukemia at the time of diagnosis present with increased serum levels of sTNF-RII and IL-1ra. Tumor load and severity of hypermetabolism are not associated with nutritional status. We conclude that our patients presented an early stage of malnutrition given the low mean plasma protein concentrations but normal anthropometric characteristics. There were no correlations between sTNF-RII, IL-1ra, tumor load, hypermetabolism and nutritional indices.

Both cytokine antagonists, sTNF-RII and IL-1ra, may contribute to prevent overt malnutrition from already detectable mild malnutrition since both antagonize the tissue wasting cytokines TNF-a and IL-1 and thus counteract wasting and cachexia [18, 19]. A variety of stimuli trigger the shedding of sTNF-RII from the cell surface of stimulated monocytes or neutrophils into the circulation to suppress TNF-mediated responses [8]. In leukemia, sTNF-RII counteract the effect of TNF-a on the proliferation of leukemia cells [20] and prevent malnutrition. In accordance with this hypothesis higher sTNF-RII levels were significantly correlated with a high tumor load.

Il-1ra binds to the same receptors as IL-1 but is biologically inactive and therefore a competitive inhibitor of IL-1 [9]. IL-1ra mainly inhibits leukemia cell proliferation in-vitro [21]. We found an even lower mean serum level in the group with a high tumor load than in the group with a low tumor load. Similarly, it is reported that the marrow serum of AML patients contains less IL-1ra protein than marrow serum of normal individuals [22]. Perhaps, IL-1ra is upregulated in our children to act mainly anti-inflammatory and anti-cachectic and not primarily anti-leukemic. Further studies are needed to determine the effect of the elevated serum levels of sTNF-RII and IL-1ra in leukemia on nutritional status.

The lack of an impact of tumor load and hypermetabolism on nutritional status could be due to the generally early diagnosis of acute leukemia in children before malnutrition might occur. Accordingly, mean energy expenditure in our 15 patients was not significantly increased. In other studies, measured resting energy expenditure was not different to controls in low-risk children [4, 23] in contrast to children with a high tumor load [11].

In accordance with our study, most other studies also found that children with acute leukemia do not present with overt signs of malnutrition at diagnosis when anthropometric characteristics are used for assessment [3-6]. In contrast, in a large retrospective multicenter study of 1019 children, the prevalence of malnutrition (defined as body mass index SDS ≤ 2) was 7.6% of the boys and 6.7% of the girls [1], which was approximately three times higher than expected. Only one study from Birmingham reported malnutrition in 15% of 33 leukemia children at diagnosis by arm anthropometry (mid-upper arm circumference and triceps skinfold thickness $<5^{th}$ percentile), but median indices were not different to controls [2].

Some studies proposed that anthropometric characteristics are not sensitive enough to diagnose malnutrition and suggested to evaluate patients based on plasma proteins, especially prealbumin with its short half-life [3,7]. In our study, the mean levels of prealbumin, transferrin and albumin were significantly lower in leukemia children than in controls. Yu et al. [3] found similar results in a small series of 9 children with newly diagnosed or relapsed leukemia compared to 16 children under treatment with overall normal anthropometric characteristics. These proteins are also acute-phase reactants with decreased levels during stress situations, especially during fever and infections, changes in hydration and in liver diseases, but, these factors were excluded in our patients. Thus, the low plasma protein concentrations can be attributed to mild malnutrition. Mediators may induce mild malnutrition in acute leukemia and cytokine antagonists like TNF-RII and IL-1ra may then be upregulated to counteract the mediators and thereby prevent progress to overt malnutrition.

We conclude that children with acute leukemia present with mild malnutrition irrespective of high tumor load or hypermetabolism. Both cytokine antagonists, sTNF-RII and IL-1ra, are increased, which may contribute to prevention of malnutrition.

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References

1. Reilly JJ, Weir J, McColl JH, Gibson BES: Prevalence of protein-energy malnutrition at diagnosis in children with acute lymphoblastic leukemia. J Pediatr Gastroenterol Nutr 1999; 29:194-197.
2. Smith DE, Stevens MCG, Booth IW: Malnutrition at diagnosis of malignancy in childhood: common but mostly missed. Eur J Pediatr 1991; 150:318-322.
3. Yu LC, Kuvibidila S, Ducos R, Warrier RP: Nutritional status of children with leukaemia. Med Pediatr Oncol 1994; 22:73-77.
4. Delbecque-Boussard L, Gottrand F, Ategbo S, Nelken B, Mazingue F, Vic P, Farriaux JP, Turck D: Nutritional status of children with acute lymphoblastic leukemia: a longitudinal study. Am J Clin Nutr 1997; 65:95-100.
5. Uderzo C, Rovelli A, Bonomi M, Barzaghi A, Strada S, Balduzzi A, Pirovano L, Masera G: Nutritional status in untreated children with acute leukemia as compared with children without malignancy. J Pediatr Gastroenterol Nutr 1996; 23:34-37.
6. Brennan BMD, Thomas AG: Nutritional status in children with acute leukemia. J Pediatr Gastroenterol Nutr 1997;25:248-249.

7. Kurugol Z, Egemen A, Cetingul N, Oztop S, Kavakli K, Nisli G: Nutritional status of children with leukemia (letter). Med Pediatr Oncol 1997; 28:321-322.
8. Van Zee KJ, Kohno T, Fischer E, Rock CS, Moldawer LL, Lowry SF: Tumor necrosis factor soluble receptors circulate during experimental and clinical inflammation and can protect against excessive tumor necrosis factor a in vitro and in vivo. Proc Natl Acad Sci USA 1992; 89:4845-4849.
9. Dinarello CA: Biologic basis for interleukin-1 in disease. Blood 1996; 87:2095-2147.
10. Strassmann G, Kambayashi T: Inhibition of experimental cancer cachexia by anti-cytokine and anti-cytokine-receptor therapy. Cytokine and Molecular Therapy 1995; 1:107-113.
11. Stallings VA, Vaisman N, Chan HSL, Weitzman SS, Hahn E, Pencharz PB: Energy metabolism in children with newly diagnosed acute lymphoblastic leukemia. Pediatr Res 1989; 26:154-157.
12. Fleish, A: Le metabolism basal standard et sa determination an moyen du "Metabocalculator". Helv Medica Acta 1951; 18:23.
13. Schofield, WN: Predicting basal metabolic rate, new standards and review of previous work. Hum Nutr Clin Nutr 1985; 39 (Suppl 1):5-41.
14. Boothby WM, Berkson J, Dunn HL: Studies of the energy of metabolism of normal individuals: a standard for basal metabolism with a normogram for clinical application. Am J Physiol 1936; 3:468-483.
15. Prader A, Lasrgo RH, Molinari L, Issler C: Physical growth of Swiss children from birth to 20 years of age. First Zurich longitudinal study of growth and development. Helvetica Paediatrica Acta 1988; 43 (Suppl 52):1-125.
16. Gibson RS, editor. Nutritional Assessment. A laboratory manual. New York, Oxford; Oxford University Press; 1993, 60p.
17. Houtkooper LB, Going SB, Lohman TG, Roche AF, Van Loan M: Bioelectrical impedance estimation of fat-free body mass in children and youth: a cross-validation study. J Appl Physiol 1992; 72:366-373.
18. Laviano A, Gleason JR, Meguid MM, Yang ZJ, Cangiano C, Rossi Fanelli F: Effects of intra-VMN mianserin and IL-1ra on meal number in anorectic tumor-bearing rats. J Investig Med 2000; 48:40-48.
19. Teng MN, Turksen K, Jacobs CA, Fuchs E, Schreiber H: Prevention of runting and cachexia by a chimeric TNF receptor-Fc protein. Clin Immunol Immunopathol 1993; 69:215-222.
20. Carter A, Haddad N, Draxler I, Israeli E, Raz B, Tatarsky I: Respective effects of soluble interleukin-1 receptor and tumor necrosis factor receptor on IL-1 and TNF-alpha-induced DNA synthesis of common acute lymphoblastic leukemia blasts in vitro. Br J Haematol 1994; 86:22-29.
21. Stosic-Grujici S, Basara N, Dinarello CA: Modulatory in vitro effects of interleukin-1 receptor antagonist (IL-1ra) or antisense oligonucleotide to interleukin-1 beta converting enzyme (ICE) on acute myeloid leukaemia (AML) cell growth. Clin Lab Haematol 1999; 21:173-185.
22. Tao M, Li B, Nayini J, Andrews CB, Huang RW, Devemy E, Song S, Venugopal P, Preisler HD: SCF, IL-1 beta, IL-1ra and GM-CSF in the bone marrow and serum of normal individuals and of AML and CML. Cytokine 2000; 12:699-707.
23. Mauer AM, Burgess JB, Donaldson SS, Rickard KA, Stallings VA, van Eys J, Winick M: Special nutritional needs of children with a malignancy: a review. J Parenter Enteral Nutr 1990; 14:315-324.

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