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Original Paper

Restorative Effect of Total Parenteral Nutrition on Natural Killer Cell Activity in Malnourished Cancer Patients

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Decreased natural killer cell activity (NKCA) is associated with malnutrition in both cancer and non-cancer patients. We have studied the effect of total parenteral nutrition (TPN) on NKCA in 9 malnourished cancer patients, candidates for surgery. TPN was administered for a median of 10 days (range 7 – 11), providing 1.5-fold the estimated resting energy expenditure, with 30% as fat. Calorie:nitrogen ratio was 150:1. Basal human recombinant interferon-α2a (rIFN-α2a) and human recombinant IL-2 (rIL-2) activated NKCA were measured, as were the main nutritional parameters, prior to and after TPN. NKCA increased in all patients and reached the normal range in 5, 3 and 4 subjects, respectively, for basal, rIFN-α2a and rIL-2 activated NKCA. As regards nutritional assessment, body weight and IgM levels significantly increased from 47.7 to 50.1 kg and from 174 to 237 mg/dl, respectively. This study demonstrates that a 10-day TPN course increases and sometimes restores normal NKCA. Such effect was constant and preceded nutritional changes.

Key words: natural killer cell, malnutrition, cancer, total parenteral nutrition
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INTRODUCTION

DEPRESSED NATURAL killer cell activity (NKCA) has been reported in children with chronic protein calorie malnutrition [1–3], as well as in patients with a variety of solid tumours [4, 5]. We have recently confirmed these findings in malnourished cancer patients [6], and have investigated whether the depressed NKCA could be restored in these patients by adequate nutritional support.

The issue needs to be urgently investigated, especially since the infusion of triglycerides, an essential component of modern regimens of total parenteral nutrition (TPN), has been reported to be associated with higher risks of infection, both in experimental [7–9] and clinical [10, 11] settings. Most studies have explored different aspects of the immune response of the host, and have mainly focused on neutrophil function [12–15], monocyte/macrophage function [16, 17], reticuloendothelium system function [18–20], the complement system [12, 13, 16, 17], and humoral [12, 17, 21] and cellular immunity [6, 7, 12, 13, 21–25]. Data regarding natural killer cells are particularly scarce and deal with changes in the number of these cells rather than the cytotoxic activity of this lymphocytic subpopulation.

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The aim of this study was to investigate the effect of a 10-day course of TPN on NKCA in malnourished cancer patients.

PATIENTS AND METHODS

Patients

From May 1989 to January 1991, 9 malnourished cancer patients, candidates for radical or palliative surgical procedures, were studied at the Istituto Nazionale Tumori (Milan, Italy). All patients had shown weight loss higher than 10% of their usual body weight in the preceding 6 months, and the cancer diagnosis was histologically proven.

We performed a nutritional assessment by anthropometric, biochemical and haematological parameters of nutritional status, and an immunological assessment before and after an average of 10 days of TPN (range 7–11).

The nutritional regimen was planned at 1.5-fold the resting energy expenditure, estimated by the Harris–Benedict equation [26]. The non-protein calorie source included glucose and fat (Intralipid 20%; Kabi Pharmacia AB, Stockholm, Sweden) covering 70% and 30% of the energy intake, respectively. The calorie:nitrogen ratio was 150:1. The protein source was supplied by a free amino acid solution (Freamine III; Baxter, Kendall McGraw Laboratories Inc., Irvine, California, U.S.A.). Electrolytes, vitamins and trace elements (Zn, Cu, Mn, Cr) were given according to current recommendations [27].

The TPN mixture was delivered through a central venous catheter in a subclavian vein, using an ethyl-vinyl-acetate (E.V.A.) "all-in-one" bag, while only vitamins were infused by a separate line (Idroplurivit—Menarini, Italy; 1 f/day). Vitamins were as follows: B1 3.2 mg; B2 3.2 mg; pantothenic acid 5 mg; niacin 32 mg; C 100 mg; A 16.600 IU; D2 3.300 IU; E 8 mg.

The characteristics of the patients are reported in Table 1 [28–30].

No patient previously underwent any radiation therapy or chemotherapy, and no metabolic or immunological disease was detected.

Preparation of effector cells

Peripheral blood mononuclear cells (PBMC) were separated from heparinised blood samples by density centrifugation on Ficoll-Hypaque gradients (Pharmacia Fine Chemicals, Uppsala, Sweden). The separated cells were washed twice in Hank's solution (Gibco, Grand Island, New York, U.S.A.) and the number of viable cells was determined by a trypan blue exclusion test. Cells were resuspended in RPMI 1640 containing 1% glutamine (Gibco).

Target cells

K562 cells (human myelogenous leukaemia with haematogenic potential) were cultured in suspension in RPMI 1640 plus 10% FCS (fetal calf serum) and labelled by exposure for 1 h to 100 μCi $Na^{51}CrO_4$.

Cytotoxicity assay

NKCA was assessed in an 18-h ^{51}Cr release assay by adding 3×10^3 target cells in 0.1 ml RPMI to 0.08 ml of effector cells at varying concentrations (50:1, 25:1, 12:1, 6:1 [31]) to obtain the desired final effector-to-target cell ratios.

All assays were carried out in triplicate, in flat-bottom wells of a microtitre plate (CoStar, Cambridge, Massachusetts, U.S.A.) in a final volume of 0.2 ml. The plates were incubated for 18 h at 37°C in a humidified 5% CO_2 incubator. In order to harvest the cells, the plates were centrifugated at 450 *g* for 5 min and 0.1 ml of supernatant was removed for counting. Spontaneous release was evaluated by omitting effector cells, and maximum release was determined by incubating targets in 2 N HCl, which releases 75–95% of total counts. Per cent cytotoxicity was calculated as follows:

$$\frac{\text{c.p.m. experimental} - \text{c.p.m. spontaneous} \times 100}{\text{c.p.m. maximum} - \text{c.p.m. spontaneous}}$$

Human recombinant interleukin-2 (rIL-2) and human recombinant interferon-α 2a (rINF-α2a) activated cytotoxicity

A total of 2×10^6 PBMC in 1 ml of RPMI were incubated for 1 h at 37°C with 1000 U of rIFN-α2a (Roche, Basel, Switzerland). When treated with rIL-2 (Biogen, Cambridge, U.S.A.), 2×10^6 PBMC in 1 ml of RPMI were incubated at 37°C overnight with 600 U of rIL-2. Treated PBMC were then

Table 1. *Patients' characteristics*

Patient	Sex	Age (years)	Diagnosis (stage)	Weight (kg)	Body mass index	% Weight loss	Triceps skinfold percentile (mm)*	Arm circumference percentile (cm)*	RBP (mg/dl)	Cholinesterase (mU/ml)
1	M	53	Gastric cancer stage IV	65	20	10	25 (8)	5 (26)	1.2	1795
2	F	48	Rectal cancer Dukes' D	40	16	17	7.5 (9)	5 (21)	–	2321
3	F	53	Gastric cancer stage III	42	15	19	5 (4)	5 (19.5)	3.7	1623
4	M	60	Gastric cancer stage III	42	15	11	5 (4)	5 (19)	3.4	1592
5	F	77	Gastric cancer stage II	35	15	20	5 (7)	5 (18)	2.1	1027
6	F	69	Breast cancer stage IV	48	18	16	5 (9)	5 (22)	2.8	2065
7	M	60	Gastric cancer stage III	64	22	22	60 (12)	25 (29)	3.3	1469
8	F	49	Cancer of pancreas stage IV	41	15	30	7.5 (5.5)	5 (18.5)	2.9	1400
9	M	75	Colon cancer Dukes' D	52	19	32	10 (6)	5 (25)	4.2	2035

TNM classification [28] and Dukes' classification [29] were employed for staging neoplastic disease.
RBP, retinol binding protein, normal range 3–6 mg/dl; cholinesterase normal range 1900–3800 mU/ml *Reference [30].

tested for cytotoxicity in an 18 h ^{51}Cr release assay, as described above.

Statistical analysis

Changes in the nutritional and immunological variables during TPN were analysed by paired Student's *t*-test, and 95% confidence intervals on the difference between the means of the samples are reported.

NKCA of the patients was compared with that of a control group of 37 healthy subjects, aged from 25 to 67 years, who are usual blood donors of our institute. The normal NKCA level was calculated as their mean value, with the lower limit as the mean −2 S.D.

RESULTS

All the patients included in this study completed it. We did not observe any complications related either to parenteral nutrition or to the central venous catheters.

The nutritional status of all patients was poor and characterised by a mean weight loss of 19.6% of the usual body weight, a low body mass index (B.M.I.) (mean 17.2), low triceps skinfold (mean, 14th percentile) and arm circumference (mean, 7th percentile). Serum retinol-binding-protein (RBP) and serum cholinesterase were reduced and serum albumin was at the minimum of the normal range (Table 1).

Comparing NKCA of the patients prior to TPN with normal levels, we observed a low value for all patients (Figure 1).

Table 2 summarises the effects of TPN on nutritional status and usual immunological indexes. We observed a significant increase in only mean body weight and mean IgM level, from 47.7 to 50.1 kg ($P = 0.02$) and from 174 to 237 mg/dl ($P = 0.02$), respectively.

The influence of TPN on NKCA is shown in Table 3. TPN significantly increased the mean basal NKCA from 36 to 55, the mean rIFN-α2a stimulated NKCA from 53.8 to 70.4, and the mean rIL-2 stimulated NKCA, from 69.0 to 79.9.

For basal NKCA and rIFN-α2a-stimulated NKCA, the mean values after TPN were above the lower limit of normality (55.0 versus 52.3 and 70.4 versus 69.8, respectively) and rIL-2 NKCA almost reached it (79.9 versus 81.9). In particular, 5 patients' basal NKCA, 3 patients' rIFN-α2a-stimulated NKCA and 4 patients' rIL-2-stimulated NKCA were normalised after TPN, and all the other patients had increased NKCAs (Figure 1).

Table 2. Main nutritional characteristics of the series tested before and after total parenteral nutrition (TPN)

	Before TPN	After TPN	*P*	95% CI
Weight (kg)	47.7	50.1	0.02	0.4–4.5
Triceps skinfold (mm)	7.2	7.7	n.s.	
Arm circumference (cm)	22.0	22.0	n.s.	
Arm muscle circumference (cm)	19.7	19.6	n.s.	
Albumin (n.v. 3.5–4.8 g/dl)	3.5	3.4	n.s.	
Total protein (n.v. 6–8 g/dl)	6.2	6.2	n.s.	
Pre-albumin (n.v. 10–40 mg/dl)	19.2	21.0	n.s.	
Retinol binding protein (n.v. 3–6 mg/dl)	2.9	3.6	n.s.	
Cholinesterase (n.v. 1900–3800 mU/ml)	1703	1523	n.s.	
IgA (n.v. 150–400 mg/dl)	244	294	n.s.	
IgG (n.v. 800–1800 mg/dl)	1150	1277	n.s.	
IgM (n.v. 60–280 mg/dl)	174	237	0.02	9–75
C_3 (n.v. 55–120 mg/dl)	94	102	n.s.	
C_4 (n.v. 21–49 mg/dl)	53	46	n.s.	
Total iron-binding capacity (n.v. 250–400 mg/dl)	279	254	n.s.	
Lymphocytes (no/mm^3)	1515	1426	n.s.	

All the values are reported as means.
n.v., normal values of our laboratory; n.s., not significant; 95% CI, confidence intervals on difference between the means of the samples.

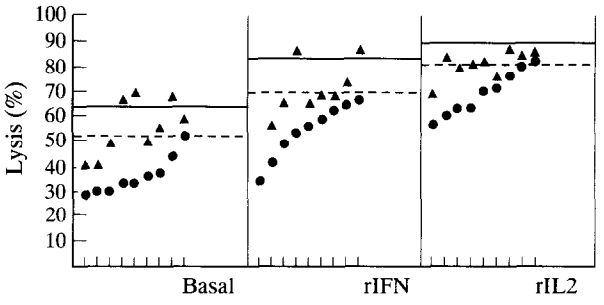

Figure 1. Basal natural killer cell activity (NKCA), recombinant interferon-α2a (rIFN-α2a) and recombinant interleukin-2 (rIL-2) stimulated NKCA for each subject before (●) and after (▲) total parenteral nutrition. The solid horizontal lines represent the mean value of healthy subjects (blood donors, 64.9, 83, 89.9 for basal NKCA, rIFN-α2a and rIL-2 stimulated NKCA, respectively) and the broken lines represents the lower limit of normality, calculated as the mean −2 S.D. (52.3, 69.8, 81.9 for basal NKCA, rIFN-α2a and rIL-2 stimulated NKCA, respectively). NKCA is expressed as percentage of lysis at an effector:target ratio of 50:1.

Table 3. Natural killer cell activity (NKCA) before and after 10 days of total parenteral nutrition

	NKCA expressed as % lysis					
	Basal		rIFN-α2a		rIL-2	
Patient no.	Before	After	Before	After	Before	After
1	36	50	49	65	60	83*
2	30	49	66	86*	71	75
3	33	66*	53	85*	63	80
4	28	40	56	64	82*	84*
5	37	55*	59	68	76	86*
6	34	69*	34	69	70	81
7	52	59*	62	68	80	83*
8	30	40	41	56	56	68
9	44	67*	64	73*	63	79
Mean	36.0	55.0	53.8	70.4	69.0	79.9
95% CI	11.5 – 26.5		8.5 – 27.7		5.5 – 16.3	
P	0.0003		0.001		0.001	
Normal lower limit	52.3		69.8		81.9	

Basal, basal NKCA; rIFN-α2a, NKCA after stimulation by recombinant interferon-α2a; rIL-2, NKCA after stimulation by recombinant interleukin-2; 95% CI, 95% confidence intervals for difference between the means of the samples. *Values above normal range calculated as mean value of healthy (blood donors) subjects, the lower limit as the mean −2 S.D.

DISCUSSION

Natural killer cells are a subpopulation of lymphocytes spontaneously cytotoxic against a variety of tumour cells and virus-

infected cells, gram-negative bacteria, parasites and yeasts *in vitro*. It is suggested that these cells might be involved *in vivo* in immunosurveillance of tumours and resistance to infections.

Our data show that malnourished cancer patients have a depressed NKCA compared to healthy controls (blood donors), and that basal rIFN-α2a or rIL-2-stimulated cytotoxicity is restored by a 10-day course of TPN. NKCA increase is precocious and precedes every nutritional biochemical change.

Whereas it is true that it is quite difficult to demonstrate a real nutritional benefit in these patients, since body weight increase may simply reflect an extracellular volume expansion, it is noteworthy that no nutritional deterioration occurred, as would be expected in these patients [32] if not supported.

Furthermore, TPN provided them all the glycolytic and oxidative metabolic substrates which are sources of effector cell energy [33,34], as well as micronutrients such as vitamin D [35,36], magnesium, calcium, zinc and copper, which are known to modulate the immune system function [37–46], and to be usually deficient in conditions of nutritional deprivation [47].

Regarding the controversial role of long-chain triglycerides (LCT) on NKCA, our findings are in keeping with data in animals showing a significant increase in NKCA [48] with 10% Intralipid and human data of Yamashita and associates [49] and Bray and Brahmi [50] who reported the permissive/adjuvant role of soy bean emulsions and products of lipoxygenase on NKCA.

Our results are in contrast with data reported by Monson and colleagues [22] and Seidner and colleagues [18]. However, these studies are not strictly comparable as many of the patients included in them were not malnourished and hence the reduced NKCA was probably not dependent on a deficiency of nutrients, but on the presence of the tumour itself. Different duration of TPN, as well as potential differences in the concentration of aromatic amino acids, tyrosine, phenylalanine and tryptophan and their final ester derivates, that are inhibitors of NKCA [51,52], or branched chain amino acids, that maintain immunocompetence [53], or thiol compounds (cystine or precursors) [54], which mediate human natural killer cells' proliferation, could account for the different results. It should also be noted that investigations by Monson and associates [22] and Seidner and associates [18] did not report a reference value for healthy subjects for which to compare the basal data of cancer patients prior to TPN, nor did they specify the confidence intervals of their data, so that a proper interpretation of the results is difficult.

Furthermore, the findings of a significant increase in NKCA after rIL-2 stimulation argue against the possible interference of LCT in the interaction between IL-2 and cellular receptors, as suggested by Seidner and associates [18].

The practical conclusion of this study is that, whenever malnutrition is present in cancer patients, depressed NKCA would consequently be reversed by an appropriate regimen of TPN [55].

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