Total Cell-Associated Zn^{++} and Cu^{++} and Proliferative Responsiveness of Peripheral Blood Mononuclear Cells From Patients on Chronic Hemodialysis

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We investigated total copper (Cu^{++}) and zinc (Zn^{++}) content in plasma and peripheral blood mononuclear cells (PBMC) and its impact on proliferative ability of the latter in patients on chronic hemodialysis versus age- and sex-matched healthy volunteers. Plasma levels of Cu^{++} and Zn^{++} were significantly lower in dialysis patients compared with the control group (83.6 ± 7.29 *v* 95.1 ± 9.63 μg/dL, *P* < .03 for Cu^{++}; 71.1 ± 7.64 *v* 89.7± 12.55 μg/dL, *P* < .005 for Zn^{++}). Basal total PBMC-associated Cu^{++} content was significantly higher in uremic patients (19.3 ± 3.59 *v* 14.6 ± 2.72 μmol/mg protein, *P* < .005). Basal PBMC-associated Zn^{++} concentration was also significantly elevated in hemodialysis patients compared with their healthy counterparts (23.9 ± 5.64 *v* 10.5 ± 2.64 μmol/mg protein, *P* < .005). In addition, we incubated PBMC of the uremic patients versus healthy control PBMC in a Zn^{++}-free versus Zn^{++}-enriched medium. After a 72-hour incubation, total cell-associated Zn^{++} of both normal and uremic cell populations increased significantly compared with the respective baselines (34.6 ± 22.49 *v* 4.3 ± 1.42 and 20.3 ± 10.71 *v* 5.8 ± 2.22 μmol/mg protein, respectively). However, no statistically significant difference was evident between the 2 groups (34.6 ± 22.49 *v* 20 ± 10.7 μmol/mg protein). Total cell Zn^{++} content, on the other hand, was significantly increased in uremic PBMC after 72 hours of incubation in Zn^{++}-enriched medium compared with the control group (63.3 ± 26.12 *v* 18.6 ± 13.42 μmol/mg protein, *P* < .005). A significant increase in PBMC proliferation evaluated by ^{3}H-thymidine incorporation was evident in the Zn^{++}-enriched culture (35,559 ± 4,136 counts per minute [CPM] *v* 20,497 ± 7,263 CPM, *P* < .005). Cu^{++} enrichment of the medium, while resulting in a modest elevation of cell-associated Cu^{++}, did not produce such a proliferative effect.

PATIENTS WITH END-STAGE renal failure often have severe trace metal deficiency, which is not corrected by dialysis.[1,2] Trace metals, including zinc (Zn^{++}) and copper (Cu^{++}), are directly involved in metabolic processes critical to cell differentiation and replication.[3-7] Because many immunologic functions depend on these processes, Cu^{++} and Zn^{++} are believed to be essential to functioning of immunocompetent cells residing in circulating blood, and their deficiency may result in impairment of immune responsiveness.[1-7]

Immunodeficiency is a well-documented consequence of chronic renal failure.[8-14] Several in vivo clinical studies are relevant to this issue. Thus, addition of Cu^{++} or Zn^{++} to the diet and, in some cases, to the dialysate of patients on chronic hemodialysis or on peritoneal dialysis significantly improved various immunologic functions and proliferation of peripheral blood mononuclear cells (PBMC), along with moderate elevation of plasma Cu^{++} or Zn^{++} level.[15–18]

However, plasma Cu^{++} or Zn^{++} concentrations are by no means reliable indices of the state of their total body stores. Those major and trace elements, which are mainly located intracellularly, may appear within normal range in plasma concomitantly with depleted intracellular stores.[8-22] For this reason, the cell metal content is considered a more appropriate indication of total body status. It is plausible that improvement of immunologic impairments after dietary supplementation of Cu^{++} and/or Zn^{++}[15-18] results from significant changes in total cell-associated Cu^{++} or Zn^{++} content rather than insignificant plasma elevation of the latter.

In the present study, we initially determined Zn^{++} and Cu^{++} concentrations in plasma of hemodialysis patients compared with normal controls. In addition, free versus ceruloplasmin bound copper fractions were assessed in both experimental groups. To evaluate copper-dependent enzymatic activity, superoxide dismutase (SOD) was also measured. Assuming that PBMC-associated Zn^{++} or Cu^{++} reflects the respective state of body stores, we determined these parameters in PBMC from patients on chronic hemodialysis versus healthy controls. In addition, in cell culture assays, PBMC from the same experimental groups were incubated in media supplemented with Zn^{++} or Cu^{++} in concentrations, which proved stimulatory to cell proliferation in our preliminary experiments. Subsequently, total cell content of Zn^{++} or Cu^{++} was measured in PBMC from both populations cultured for 72 hours and was compared with mitogen-induced proliferative rates of these cells.

PATIENTS AND METHODS

Patients

A total of 25 patients on chronic hemodialysis were included in this study. Their clinical data are listed in Tables 1 and 2. Blood samples from 10 of the patients (Table 1) were used in plasma experiments. Blood samples from the other 15 (Table 2) served as a source of PBMC. Care was taken to include in the study only patients who were on chronic hemodialysis for more than 2 years, not suffering from any intercurrent infections, immunologic disorders, or taking any immunosuppressive drugs at the time of the study. Twenty-five age- and sex-matched healthy volunteers served as a control group.

Plasma Experiments

A total of 5 mL heparinized blood was drawn in the morning from 10 patients and 10 matched controls and centrifuged at 6,000 rpm. Plasma was separated from the erythrocyte pellets, which were stored in 100 μL triplicates at -80°C, to be subsequently used for SOD determination. Plasma samples were stored at -30°C for total plasma

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Submitted December 10, 1999; accepted September 18, 2000.

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0026-0495/01/5003-0003$35.00/0
doi:10.1053/meta.2001.21016

Zn^{++} and Cu^{++} determination, as well as ceruloplasmin and ceruloplasmin-bound versus free plasma Cu^{++} measurements.

Total plasma Zn^{++} and Cu^{++} measurements. Total plasma Zn^{++} and Cu^{++} concentrations were measured on an atomic absorption spectrophotometer. In brief, 1 mL of plasma was diluted in 5 mL matrix diluent prepared from 12.5 g La oxide dissolved in 200 mL concentrated hydrochloric acid (HCl) and diluted to 2.5 L in water distilled by reverse osmosis. Standard curves were prepared from 1 g/L stock solutions of $CuCl_2$ or $ZnCl_2$ using the same matrix diluent.

SOD determination. The erythrocyte pellets were washed 3 times in phosphate-buffered saline (PBS) and lyzed in 300 mL distilled water. SOD activity was assessed using a commercially available kit (Ranox Laboratories, Ardmore, England).

Hemoglobin (Hb) concentrations were determined in each sample. The results were presented as SOD units/glycosylated hemoglobin (HBA_{1C}). All measurements were performed on a Cobas-Mira autoanalyzer (Roche, Switzerland).

Ceruloplasmin evaluation. Total plasma ceruloplasmin was determined by a standard procedure using ARRAY Systems reagent kit (Fullerton, CA) on a Beckman autoanalyzer (Beckman Instruments, Brea, CA).

Assesment of ceruloplasmin-bound and free Cu^{++} fractions. Free versus ceruloplasmin-bound copper was determined using a specific goat antihuman ceruloplasmin antibody (Sigma, St Louis, MO). In brief, an excessive amount of the antibody (5 mg/mL instead of the recommended maximal 2 mg/mL) was added to each 1 mL sample of plasma. The precipitate was separated from the supernatant and digested in 1 mL concentrated hydrochloric acid (HCl). Ceruloplasmin was once more assessed in the supernatant using ARRAY Systems reagent kit. No traces of ceruloplasmin were detected in the supernatant, indicating that the specific antibody entirely precipitated all of the ceruloplasmin in the plasma.

Total Cu^{++} content of the supernatant, as well as of the precipitate, was measured on an atomic absorption spectrophotometer. Subsequently, percentages of free and ceruloplasmin-bound Cu^{++} were calculated.

Cell Studies

PBMC procurement. For PBMC procurement, 10 mL of heparinized blood was drawn in the morning before the dialysis treatment. The cells were isolated by a standard procedure on Ficoll-Hypaque. Cell count was performed using 4% glacial acetic acid (Tűrk solution) in a hemocytometer. Cell viability was assessed by 0.1% eosin exclusion, and only cultures with viability not less than 98% were included in the study.

Experimental design. Cells from 10 patients (see Table 1) and 10 matched controls were used to establish basal total cell-associated Cu^{++} and Zn^{++} concentrations in PBMC. The PBMC from the remaining 15 samples of patients (see Table 2) and 15 matched controls were seeded in 24-well tissue culture plates, 1×10^6 cells per well, in 1 mL RPMI 1640 supplemented with fetal calf serum (FCS) and antibiotic mixture in quadriplicates. The quadruplicates were maintained as follows: (1) control untreated cultures: only phytohemagglutinine P (PHA) added to the cells at a final concentration of 10 μg/mL; (2) cells with 10 μg/mL PHA and $CuCl_2$ added to the medium at the final Cu^{++} concentration of 60 μmol/L; (3) cells with 10 μg/mL PHA and $ZnCl_2$ added to the medium at a final Zn^{++} concentration of 80 μmol/L.

The respective above-mentioned concentrations of Cu^{++} or Zn^{++} in the media were determined in preliminary experiments.

In these preliminary experiments, dose response curves for both elements were constructed within a 20 μmol/L to 200 μmol/L range. Optimal concentrations of 60 μmol/L Cu^{++} and 80 μmol/L Zn^{++}, providing maximal proliferative rate with no toxic effect on the culture as established by 0.1% eosin exclusion, were chosen for the experiment.

The proliferative assay. A total of 10 μg/mL of the mitogen PHA was added to the wells to stimulate the PBMC proliferation. The cells were maintained in a humid incubator with 5% CO_2 at 37°C for 72 hours. A total of 1 μCi/mL ^{3}H–thymidine was added to 2 wells of each quadruplicate 24 hours before the end of the 72-hour culture. The cells from the ^{3}H-thymidine pulsed wells were collected into polystyrene test tubes and the excessive radioactive material washed out by sequential centrifugations in PBS, pH7.4. The proliferation rate of the remaining cell pellets was evaluated by ^{3}H-thymidine incorporation; the radioactivity of the samples was measured in a beta counter (Packard, Downers Grove, IL). The results were presented in counts per minute (CPM).

Total cell-associated Cu^{++} and Zn^{++} evaluation. PBMC Cu^{++} and Zn^{++} content were measured by atomic absorption spectrophotometry. Ten micrograms of suspension were allocated to be used for protein determination by Bradford assay using Coomassic Blue dye and bovine serum albumin for preparation of standard solutions.[23] The mononuclear cell samples were then digested in 1 mL concentrated HCl. Subsequently, 300 mL of digested PBMC were placed in polystyrene test tubes. A total of 5 mL of matrix diluent was added to each tube and the procedure performed as described earlier. The results were reported as μmol/L Cu^{++} and Zn^{++} per mg protein.

Data Presentation and Statistical Analysis

The results are presented as means ± SD of 10 experiments (n = 10) for plasma and PBMC basal Cu^{++} or Zn^{++} levels and 15 experiments (n = 15) for radioactive CPM and for Cu^{++} or Zn^{++} concentrations after 72 hours of incubation in Cu^{++}- or Zn^{++}-enriched medium. The differences between the results were evaluated by Kruskal-Wallis analysis of variance (ANOVA) test using Epistat 3 (1991) program.

RESULTS

The clinical data of the patients participating in the study are listed in Tables 1 and 2. Table 3 shows the data concerning red blood cell (RBC) SOD activity, serum ceruloplasmin, ceruloplasmin-bound Cu^{++}, and free serum Cu^{++}.

As can be seen, no significant difference is evident between SOD activity of RBC from patients versus normal controls (1,274.9 ± 278.27 *v* 1,218.6 ± 163.79, $P = .63$, n = 10). In addition, absolute values of ceruloplasmin (42.24 ± 3.65 *v*

Table 1. Clinical Data on Hemodialysis Patients Participating in the Experiments Performed on Plasma

No.	Years on Hemodialysis	Primary Disease	Age (yr)/Sex
1	3	HTN	86/F
2	2	HTN, CHR PN	71/M
3	2	PKD, HTN	63/F
4	2	Unknown	75/F
5	4	NS	71/M
6	4	HTN	74/M
7	1	PKD, HTN	73/M
8	6	HTN, NL	77/M
9	7	HTN, CHR GN	63/M
10	3	Unknown	89/M
Mean	4		74.2

Abbreviations: HTN, hypertension; CHR PN, chronic pyelonephritis; PKD, polycystic kidney disease; NS, nephrotic syndrome; NL, nephrolithiasis; CHR GN, chronic glomerulonephritis; F, female; M, male.

Table 2. Clinical Data on Hemodialysis Patients Serving as the Source of PBMC

No.	Years on Hemodialysis	Primary Disease	Age (yr)/Sex
1	6	CHR PN	86/F
2	3	HTN	54/M
3	2	HTN	40/F
4	2	PKD, HTN	45/F
5	4	HTN	73/F
6	3	CHR GN	51/M
7	15	CHR GN, REC UTI	49/F
8	6	PKD	67/M
9	3	REF NEPHR	28/M
10	7	HTN	88/M
11	7	HTN, NS	63/F
12	2	Unknown	75/F
13	5	HTN, NL	77/M
14	2	PKD, HTN	73/M
15	3	NS	71/M
Mean	6		61.6

Abbreviations: REF NEPHR, reflux nephropathy; REC UTI, recurrent urinary tract infection.

Table 3. Data on Ceruloplasmin-bound Versus Free Copper and on SOD Activity in Uremic Patients Versus Healthy Controls

No.	Test	Control Group	Patient Group	*P* Value
1	SOD activity (SD) units/gHb	1,274.9 ± 278.27	1,218.6 ± 163.79	$P = .63$ (NS)
2	Ceruloplasmin (mg/dL)	42.240 ± 3.65	35.98 ± 5.54	$P = .07$ (NS)
3	% free copper	13.74 ± 1.388	18.42 ± 3.118	$P < .02$*
4	% ceruloplasmin-bound copper	86.7 ± 1.40	81.48 ± 1.26	$P < .02$*

Abbreviation: Hb, hemoglobin.
*Significant difference ($P < .05$).

35.98 ± 5.54 mg/dL, $P = .07$, n = 10) as well as ceruloplasmin-bound or free copper values were not statistically different in the 2 groups. However, when fractional values were calculated, percent of ceruloplasmin-bound Cu^{++} was significantly lower and, consequently, percent of free serum copper was significantly higher in the patient group.

Plasma Cu^{++} and Zn^{++} values are presented in Fig 1. Zn^{++} levels in the plasma of dialysis patients were found to be significantly lower than those of normal controls (71.64 ± μg/dL *v* 89.7 ± 12.55 μg/dL, respectively, $P < .005$, n = 10).

Similar results were obtained with Cu^{++} (patients, 83.6 ± 7.29 μg/dL *v* control, 95.1 ± 9.63 μg/dL, $P < .03$, n = 10).

Figure 2 presents basal total cell-associated Cu^{++} and Zn^{++} values in PBMC from dialysis patients versus normal controls. In patient PBMC, Zn^{++} levels were significantly higher compared with normal controls (23.9 ± 5.64 μmol/mg protein *v* 10.5 ± 2.64 μmol/mg protein, $P < .005$, n = 10). Total cell-associated Cu^{++} concentrations were also slightly elevated in dialysis patients compared with the control group, although the difference did not reach statistical significance (19.3 ± 3.59 *v* 14.6 ± 2.72 μmol/mg protein, $P = .6$, n = 10).

Total cell-associated Cu^{++} and Zn^{++} concentrations in PBMC after 72 hours of incubation in Cu^{++}- or Zn^{++}-enriched medium are presented in Fig 3.

As can be seen, when cells were incubated in a medium enriched with 80 μmol/L of Zn^{++}, only a slight elevation of cell-associated Zn^{++} levels was evident in PBMC of normal controls. On the other hand, in PBMC of uremic patients, cell-associated Zn^{++} concentrations increased from 18.5 ± 4.55 μmol/mg protein to 65.3 ± 26.12 μmol/mg protein ($P < .005$, n = 15). When the cells were grown for 72 hours in a medium supplemented with 60 μmol/L Cu^{++}, total intracellular Cu^{++} levels also increased significantly in both experimental groups. No significant difference was observed between cell-associated Cu^{++} of the 2 cell populations after 72 hours of incubation in Cu^{++}-enriched medium ($P = .4$, n = 15).

Figure 4 presents the results of ^{3}H–thymidine incorporation in PBMC of the 2 experimental groups after 72 hours of

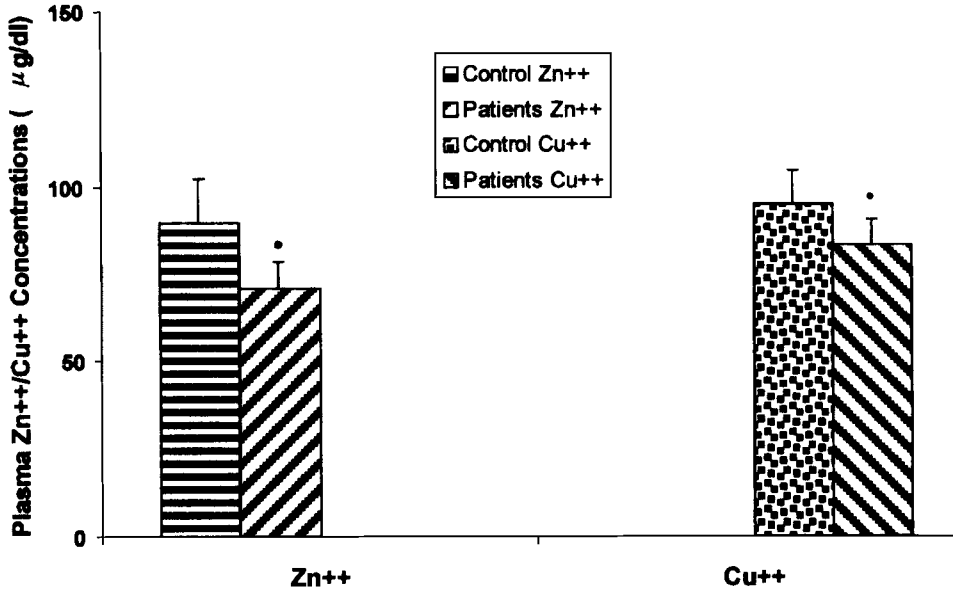

Fig 1. Basal levels of Zn^{++} and Cu^{++} in plasma from hemodialysis patients *v* normal controls. Zn^{++}, zinc (concentrations in μg/dL); Cu^{++}, copper (concentrations in μg/dL). ●, Significant difference ($P < .05$) from the respective baseline.

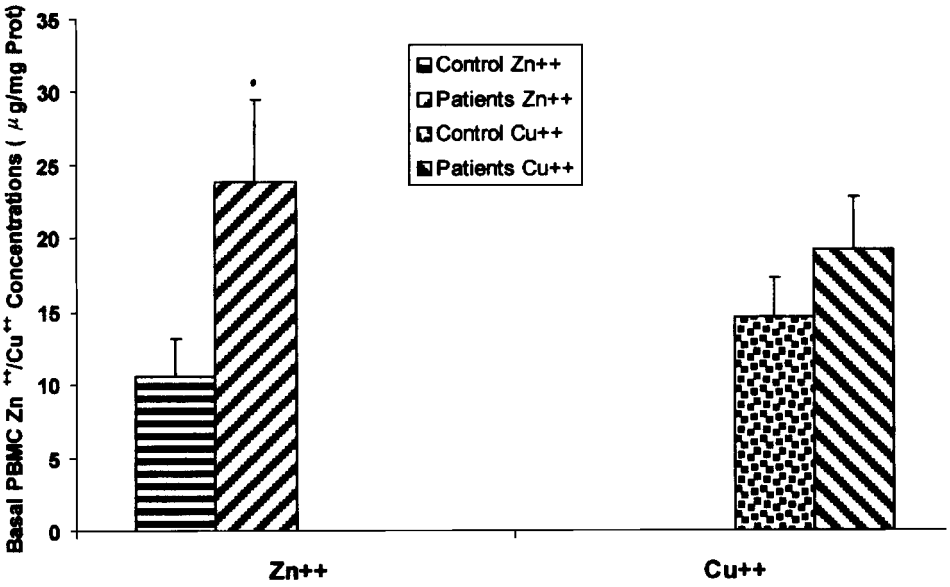

Fig 2. Basal Zn^{++} and Cu^{++} concentration in freshly procured PBMC from hemodialysis patients *v* normal controls.

PHA-stimulated cell proliferation in a Cu^{++}- or Zn^{++}-enriched medium.

As can be seen, although the addition of 80 μmol/L Zn^{++} to the culture medium produced a significant enhancement of cell proliferation in both experimental groups compared with their respective baselines, this elevation was much higher in dialysis patients (35,559 $\pm$ 4,136 CPM compared with baseline 12,046 $\pm$ 3,468 CPM, $P < .05$, n = 15) than in normal controls (20,497 $\pm$ 7,263 *v* the baseline, $P < .05$, n = 15).

The addition of 60 μmol/L Cu^{++} to the culture medium also produced a substantial increase in CPM values in PBMC from both control and hemodialysis groups, although the difference between the groups was not statistically significant. No significant direct correlation between the CPM values and total cell-associated Cu^{++} or Zn^{++} concentration was evident ($r = .024$, $P = .017$ for the patient group; $r = .025$, $P = .01$ for the control group).

DISCUSSION

In the present investigation, we have found that normal, as well as uremic PBMC, are capable of elevating total cell-associated concentrations of Cu^{++} or Zn^{++} whenever the latter are available in the cell culture medium. Furthermore, both normal and uremic PBMC responded to total cell-associated copper or zinc elevation by augmented PHA-induced cell proliferation. However, the magnitude of this effect was significantly greater in PBMC isolated from blood of patients on chronic hemodialysis.

Basic plasma levels of Cu^{++} and Zn^{++} were found signif-

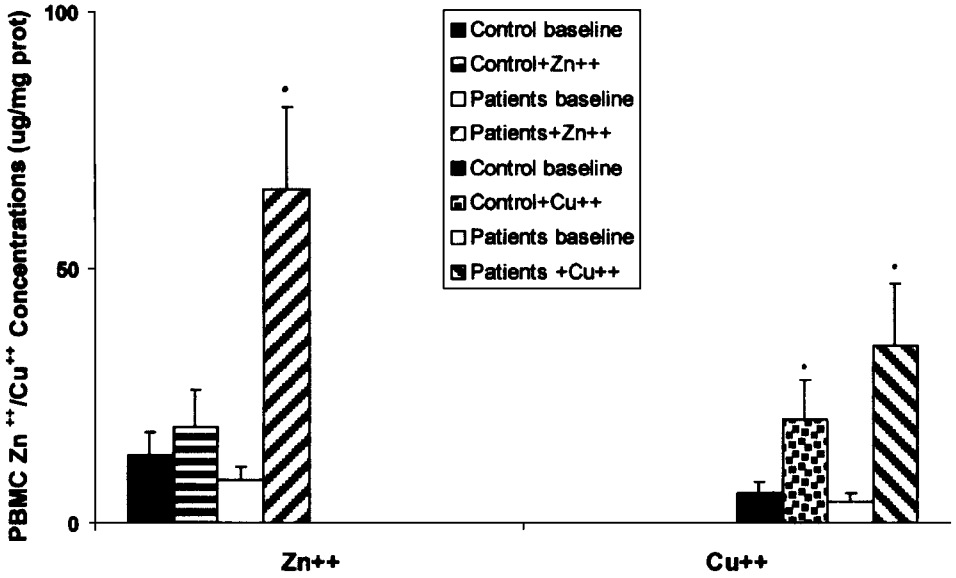

Fig 3. Zn^{++} and Cu^{++} concentrations in PBMC from hemodialysis patients *v* normal controls after 72 hours of incubation in culture medium enriched with Zn^{++} or Cu^{++}.

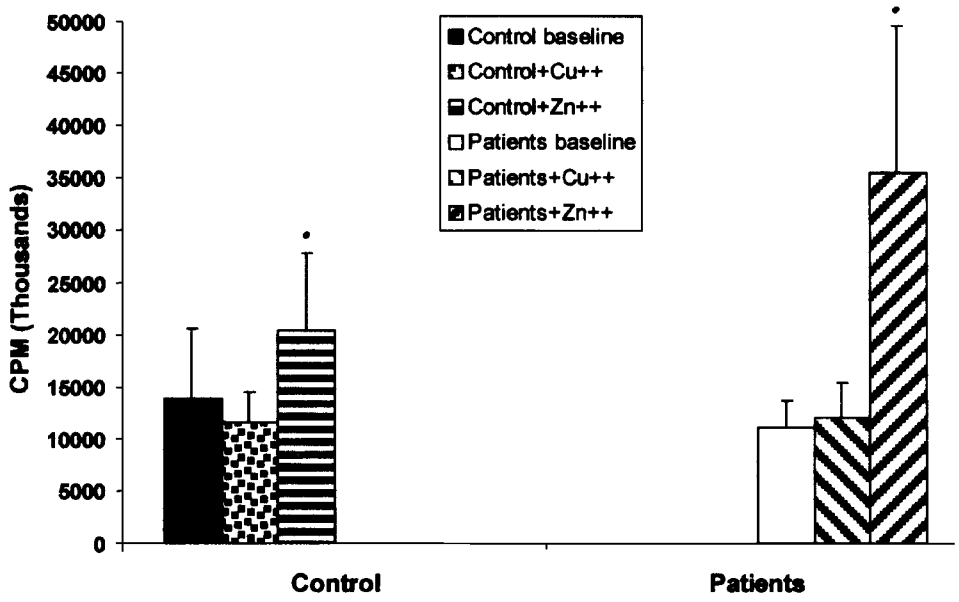

Fig 4. Proliferative rates of PBMC from hemodialysis patients *v* normal controls assessed as ^{3}H–thymidine incorporation.

icantly lower in dialysis patients compared with normal controls. These results are in keeping with a number of studies reporting lower Cu^{++} and Zn^{++} plasma concentrations in predialytic, as well as in hemo- or peritoneal dialysis-treated patients.[2,24,25] Low plasma levels of these elements cannot be attributed to or corrected by dialysis.[1-2] Plasma ceruloplasmin showed a tendency to be lower in the patient group. In addition, patient ceruloplasmin-bound copper fraction was found significantly lower compared with normal subjects. Consequently, the free copper fraction was significantly higher in the patient group. This would tend to suggest that ceruloplasmin capacity to bind Cu^{++} was decreased in uremic plasma. The mechanisms underlying these alterations in uremia, as well as the role they play in the decreased total plasma Cu^{++} concentrations[2,24,25] or the exaggerated cell-associated Cu^{++}, remain to be elucidated.

In view of these anomalies, it was interesting to evaluate the function of a typical copper-dependent enzyme, ie, SOD activity in the uremic state. This activity was not different from that of healthy controls, suggesting more complex mechanisms protecting the intergity of various enzymatic functions in the state of uremia.

As has already been mentioned, Cu^{++} and Zn^{++} are essential for a variety of metabolic processes crucial to cell differentiation and replication.[1-7,17] This is also true for cells of the immune system and, consequently, Cu^{++} or Zn^{++} deficiency usually results in impairment of immunologic functions.[15-18] It has been shown that low plasma levels of Zn^{++} are associated with impaired production of various cytokines, such as interleukin (IL)-2, IL-6, tumor necrosis factor (TNF), and interferon (INF)-γ.[26-30] This, in turn, means impaired proliferation and functioning of T lymphocytes, monocytes, natural killers, and other immunocompetent cells.[26-32] Zn^{++} deficiency results in impaired enzymatic activity of DNA-polymerase, thymidine kinase, and DNA-dependent RNA polymerase, with subsequent inhibition of DNA synthesis.[33,34] Addition of Zn^{++} restores normal IL-2 production and other immunologic cell functions.[26-36]

With respect to copper, culture of PBMC in Cu^{++}-depleted medium results in 60% to 70% inhibition of IL-2 synthesis and 40% to 70% inhibition in IL-2 messenger RNA production.[28] By limiting IL-2 production and activity, Cu^{++} deficiency severely impairs DNA synthesi.[36] Similar to Zn^{++}, the damage can be reversed by restoring normal Cu^{++} levels.[36]

Uremia is, by definition, a chronic immunodeficiency state. Impaired cytokine production, defective PBMC proliferation, susceptibility to intercurrent infections, and other expressions of severe immune deficiency have been thoroughly investigated.[8-18] Cu^{++} or Zn^{++} deficiency may play a significant role in most of these impairments. Cu^{++} or Zn^{++} supplementation in vivo in the diet of uremic patients results in a modest elevation of Cu^{++} or Zn^{++} plasma levels and subsequent improvement of immunologic deficiency.[15-18] This has been considered as additional evidence of a close relationship between the immune deficiency state of uremic subjects and their low plasma levels of Cu^{++} and Zn^{++}. Antoniou and Shalhoub[17] reported in an in vivo study that when hemodialysis patients were maintained on dietary Zn^{++} supplementation for 3 months, the proliferative responsiveness of their PBMC significantly improved. However, when PBMC of dialysis patients on regular diets were maintained in vitro in Zn^{++}-supplemented medium, they failed to show enhanced cell proliferation. In another study, the investigators added Zn^{++} to uremic cell cultures in 2 concentrations, equal to those obtained in vivo in plasma of uremic patients before and after dietary Zn^{++} supplementation, ie, not exceeding 0.1 μmol/L.[18] However, the plasma levels of either major or trace elements cannot be considered a reliable parameter for evaluation of their total body stores.[19,37] In a number of studies, PBMC were reported to more accurately reflect the total body status of these elements.[38,39] One could suggest that the significant improvement of cell proliferation after 3 months of maintaining the patients

on a Zn^{++}-enriched diet[18] probably resulted from a substantial increase in cell-associated concentrations of these elements rather than from a modest elevation in their plasma levels. Therefore, in our present investigation, we chose to compare basal total cell-associated Cu^{++} and Zn^{++} content of PBMC from hemodialysis patients versus matched normal controls with that of cells subjected to prolonged incubation in Cu^{++}- or Zn^{++}-enriched medium. The following changes in mitogen-induced cell proliferative responses were evaluated by ^{3}H-thymidine incorporation. We used 60 μmol/L Cu^{++} and 80 μmol/L Zn^{++} final concentrations in the medium, which were found to significantly stimulate PBMC proliferation in our preliminary studies. These concentrations match the previous reports on nonuremic lymphocytes in which 50 to 200 μmol/L concentrations of these elements in cell line cultures were found "mitogenic".[30,33,40]

In PBMC from dialysis patients, the total cell-associated Zn^{++} content increased dramatically after 72 hours of culture. Similarly, the consequent enhancement of PBMC proliferation in response to PHA stimulation was significantly greater compared with their normal counterparts. Nevertheless, our results also show that normal PBMC are capable of augmenting mitogen-induced proliferation in response to Zn^{++} enrichment of the medium, albeit to a lesser extent.

The addition of 60 μmol/L Cu^{++} to the cell culture medium also produced a significant increase in total cell-associated Cu^{++} of both control and uremic PBMC. This increase resulted in only a modest augmentation of mitogen-induced cell proliferaton, statistically not different between the control and the uremic groups. However, the significant increase in total cell-associated Cu^{++} in PBMC from hemodialysis patients could affect a number of other mechanisms restoring the immune responsiveness of these cells,[3] such as improvement of thymidine kinase and ribonucleotide kinase activity or normalization of RNA-polymerase functioning.[33] One could conclude that elevation of either total cell-associated Cu^{++} or Zn^{++} improves the immune responsiveness of PBMC. However, because Zn^{++} also augments production of a variety of cytokines, such as IL-1β, IL-2, IL-6, insulin growth factor, TNF, and others, its presence in the culture medium, in addition, significantly enhances cell proliferation.

Elevated concentrations of total cell-associated Cu^{++} and Zn^{++} might be a net effect of increased influx through the cell membrane on the one hand and augmented retention of these ions by the cell on the other. Elucidation of the underlying mechanism(s) was beyond the scope of this study. The simplest explanation would be that some defects in uremic cell membrane structure make the latter more permeable to Cu^{++} and Zn^{++} ions. Zn^{++} has, indeed, been found essential for cell membrane stability.[41] However, a study based on an entirely different approach is now in progress in our laboratory. The cell mitogen-induced proliferation, as evaluated by 3H-thymidine incorporation, is actually a net effect of cell division on the one hand and cell death on the other. Zn^{++} is known to play a significant role in apoptosis, the nature programmed cell death process.[42-44] Apoptosis rate is considered to be high in uremia.[38,44] Low Zn^{++} levels stimulate apoptosis in culture,[39,43] while concentrations beyond the normal culture levels inhibit apoptosis.[39] The improvement of uremic cell proliferation after incubation in a Zn^{++}-enriched environment could be, at least in part, the outcome of a modification in the apoptosis rate.

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