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ZINC CONCENTRATION IN LEUKOCYTES: MONONUCLEAR CELLS, GRANULOCYTES, T-LYMPHOCYTES, NON-T LYMPHOCYTES AND MONOCYTES*)

By

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ABSTRACT

This study was designed to evaluate the zinc concentrations of leukocytes: mononuclear cells, granulocytes, T and non-T lymphocytes and monocytes.

The zinc content of leukocytes was 0.137 ± 0.040 $\mu g/10^7$ cells. The zinc content of mononuclear cells (0.188 ± 0.030 $\mu g/10^7$ cells) was higher than in granulocytes (0.086 ± 0.023 $\mu g/10^7$ cells) ($p < 0.001$). The mononuclear cell-granulocyte ratio for zinc was approximately 2 : 1. There was no statistically significant difference between the zinc content of T lymphocytes and non-T lymphocytes. The zinc content of monocytes (0.337 ± 0.026 $\mu g/10^7$ cells) was highest in any other type of leukocyte ($p < 0.001$).

Zinc in leukocytes may, in part, influence immunity since zinc is an important part of the structure of many vital enzymes, including DNA polymerase, DNA dependent RNA polymerase and thymidine kinase, and plays a central role in cellular metabolism.

INTRODUCTION

Among the several trace metals, zinc plays an essential role in many vital enzymes, including DNA polymerase[1], DNA dependent RNA polymerase[2], and thymidine kinase[3]. Inasmuch as these enzymes are important for nucleic acid and protein synthesis and cell division, zinc appears to be essential for the integrity of host defense mechanisms.

A review of the literature failed to find any reports on the zinc content of leukocytes.

In this study we investigated the zinc content of leukocytes: mononuclear cells, granulocytes, T and non-T lymphocytes and monocytes.

MATERIALS AND METHODS

All glassware was first cleaned in the usual manner, then immersed for a minimum of 24 hours in 15 percent nitric acid, and rinsed thoroughly three times in deionized distilled water.

Eighty ml of fasting morning blood from healthy adult volunteers was drawn into heparinized disposable plastic syringes with stainless steel disposable needles.

Leukocytes were prepared by dextran sedimentation. The mononuclear cell-granulocyte ratio in the leukocyte fraction was 3 : 7. Mononuclear cells were prepared by centrifugation

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in Conray-Ficoll solution, and granulocytes were obtained from the supernatant after sedimentation of the pellet in a 3% dextran saline solution.

Contaminating erythrocytes were disrupted by hypotonic lysis. Each cell fraction was at least 95% pure. T and non-T lymphocytes were separated by a method based on the spontaneous rosette formation of human T lymphocytes with sheep erythrocytes (SRBC)[4]. Equal volumes of mononuclear cell suspension ($5\times10_6$ /m*l*) and 2% SRBC in fetal calf serum were mixed together. Mononuclear cells forming SRBC rosettes were then separated from cells not forming rosettes by centrifugation over a Conray-Ficoll gradient, as noted above. Unrosetted cells remaining at the interface and rosetted cells sedimenting to the bottom of the gradient were then removed separately; the SRBC in the pellet fraction were disrupted by hypotonic lysis, and the remaining cells were used as T lymphocytes. The interface cells were re-rosetted and centrifuged over a Conray-Ficoll layer in order to remove residual rosetting T lymphocytes. Then, monocytes containing the unrosetted cell fraction were removed by treatment with carbonyl silica. These cells were used as non-T lymphocytes. Contaminating granulocytes and monocytes were examined by Wright staining and by nonspecific esterase staining[5]. T lymphocytes in the non-T fraction were re-examined by formation of SRBC rosettes and B lymphocytes in the T fraction by a direct immunofluorescence technique using fluorescein-conjugated polyvalent goat anti-human immunoglobulin (Berringwerke AC, F. G. R). Mononuclear cells ($5\times10_6$ -1.5×10^7 /m*l*) in RPMI 1640 containing 20% fetal calf serum were incubated in a 60× 15 mm Falcon petri dish at 37°C for 12 hours.

After 4 and 12 hours of incubation, nonadherent cells were washed off. Adherent cells were removed from the plastic surface with a rubber policeman after a brief incubation on ice. Adherent cells were 83.0–97.0% monocytes.

After three washings with saline and microscopic examination, the cell pellet was suspended in 0.5 to 1.0 m*l* of deionized distilled water and disrupted by sonication.

The zinc content of these sonicated specimens was measured by flameless atomic absorption spectrophotometry using a carbon tube atomizer (Model 170-70, Hitachi, Ltd., Tokyo, Japan). Duplicate measurements were made with each sample.

RESULTS

Purity of prepared T and non-T lymphocyte fractions

As shown in table 1, T and non-T lymphocyte preparations were relatively free of other types of cells.

Zinc concentration in each cell preparation

As shown in table 2, the zinc content of leukocytes was 0.137 ± 0.040 $\mu g/10^7$ cells. The zinc concentration in mononuclear cells was higher than in granulocytes ($p<0.001$); the mononuclear cell-granulocyte ratio for zinc was approximately 2 : 1.

The zinc concentration in T lymphocytes was slightly higher than in non-T lymphocytes, but the difference was not statistically significant. The zinc content of monocytes was higher than that of any other type of leukocyte

Table 1. Contamination by other types of cells in T and non-T lymphocytes preparation

Preparation	Contaminating cells (%)		
	Granulocytes	T in non-T Non-T in T	Monocytes
T lymphocytes	3.3±1.8[a]	0.8±0.5	0.5±0.4
n=10	(0–6.2)[b]	(0–1.5)	(0–1.0)
Non-T lymphocytes	3.0±2.6	0.6±0.4	2.3±1.7
n=7	(0–5.9)	(0–1.0)	(1.0–5.0)

a Mean±1SD
b Range

Table 2. Zinc concentration in leukocytes, mononuclear cells, granulocytes, T lymphocytes, non-T lymphocytes and monocytes ($\mu g/10^7$ cells)

Leukocytes	Mononuclear cells	Granulocytes	T lymphocytes
n=9	n=11	n=13	n=10
0.137±0 040[a]	0.188±0.030	0.086±0.023	0.144±0 023
Non-T lymphocytes	Monocytes		
n=7	n=3		
0.126±0.023	0.337±0.026		

a Mean±1SD

($p<0.001$).

DISCUSSION

The present study shows differences in the zinc concentrations in different types of leukocytes in healthy adults. The zinc level in leukocytes was 0.137±0.040 $\mu g/10^7$ cells in our study. This value was reported as 0.103± 0.026 $\mu g/10^7$ cells by Fredricks et al[6], 0.193 ±0.017 $\mu g/10^7$ cells by Kumar et al[7], 0.140± 0.019 $\mu g/10^7$ cells by Dennes et al[8], and 0.152 ±0.044 $\mu g/10^7$ cell by Inutsuka et al[9]. The level reported by Kumar et al was slightly high, but our data were similar to those in the other reports.

The zinc concentration in granulocytes was 0.086±0.023 $\mu g/10^7$ cells in our study, which was higher than that reported as 0.050 $\mu g/10^7$ cells by Lennard et al[10].

When the lymphocyte population was divided into T and non-T lymphocytes, the former, with over 95% purity in the present study, had a slightly higher zinc content than the latter. The higher zinc content in monocytes, accounting for 10–30% of the total mononuclear cell number, shows that monocytes contain more zinc than T and non-T lymphocytes.

The function of zinc in the various leukocytes has not been investigated systematically. However, leukocytes contain zinc-dependent enzymes such as alkaline phosphatase and superoxide dismutase, so some of the zinc in leukocytes may be associated with the activity of these enzymes[11].

In fact, the mononuclear cell-granulocyte ratio for superoxide dismutase activity was approximately 2–3 : 1[12,13] which is in accord with the ratio for zinc (2 : 1).

Zinc induced DNA synthesis in culture of human peripheral T lymphocytes while B cells showed no response[14]. Purified T lymphocytes responded well to PHA but showed only low levels of proliferative reactivity to zinc and Con A. These diminished responses were completely restored by the addition of a small number of autologous, mitomycin-treated monocytes[14].

Recently we found significantly higher than normal zinc concentrations in lymphocytes, granulocytes, plasma and erythrocytes in a patient with Chediak-Higashi syndrome, in whom a chemotactic abnormality and a defective intracellular killing capacity were observed[15]. A marked impairment in natural killer function and in lymphocyte-mediated antibody-dependent cell-mediated cytotoxicity against tumor-cell targets has been noted in Chediak-Higashi syndrome[16,17]. On the other hand, increased natural killer cell activity and antibody-dependent cell-mediated cytotoxicity have been observed in zinc-deficient mice[18]. Thus, abnormally high zinc concentrations may be partially responsible for the impairment of some types of leukocyte function in patients with Chediak-Higashi syndrome.

Acrodermatitis enteropathica, a well-known disorder of zinc metabolism, has a high incidence of candida and bacterial infections and defects of cellular immunity such as chemotaxis, lymphocyte transformation with mitogens and a depression in T cell numbers which can be corrected by the addition of zinc[19,20]. Moreover, patients with Down's syndrome were found to have a low serum zinc level and an immune deficiency characterized by depressed neutrophil

chemotaxis, skin hypersensitivity and lymphocyte responsiveness to PHA, which were all corrected by the administration of zinc[21].

Phagocytosis, oxygen consumption and the activity of the hexosemonophosphate shunt in dog and human polystyrene latex-activated polymorphonuclear leukocytes were blocked by human prostatic fluid and prostate extract which contain 100–200 times more zinc than plasma; resting cells were not affected[22]. Extracts of dog liver, spleen and human saliva were not inhibitory, since their zinc content was not so high as that of prostatic fluid and prostate extract[22].

Thus, either deficiency or excess of zinc can interfere with functions of different cells or cell subpopulations since zinc plays a central role in celluar metabolism.

The mechanism by which zinc influences immunity is not clear. A number of factors including blockage of cell membrane receptors, changes in fluidity of membrane components, interference with the cell microskeleton such as microtubules and microfilaments, and antagonizing cations may play a pathogenetic role[23].

Further studies are needed to clarify the relationships between the function of a cell and its zinc content.

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