Zinc-Deficiency Induced Changes in the Distribution of Rat White Blood Cells

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Summary Zinc is known to play an important role for immune-functions. However, the effects of zinc-deficiency on the immune response system from the point of view of the distribution changes of the number of total white blood cells (WBCs) are still primarily unknown. Therefore, the effects of zinc-deficiency on the number of total WBCs, neutrophil, eosinophil, basophil, monocyte and lymphocytes (T lymphocyte, B lymphocyte and NK cell) were studied in rats. The weaned male rats were randomly divided into the zinc deficient diet (ZDD: 0.7 mg zinc/kg diet) group and the control diet (CON: 34.8 mg zinc/kg diet) group, and were pair-fed for 4 wk. The number of lymphocyte subsets, visceral organ weights, serum zinc, corticosterone and IL-6 concentrations were also determined. Zinc-deficiency increased duration-dependently the number of total white blood cells, granulocytes (neutrophil, eosinophil and basophil) and monocytes in 2–4 wk without changing the number of lymphocytes, T lymphocytes, B lymphocytes or NK cells. The relative weights of thymus and adrenals were 0.63 times (p<0.01) lower and 1.60 times (p<0.001) higher in ZDD group than in CON group, respectively. Zinc-deficiency increased serum corticosterone concentration to 1.48 times (p<0.05) without changing serum IL-6 concentration, as compared with those of CON group. From these results, zinc-deficiency increases markedly the number of granulocytes and monocytes without changing the number of lymphocytes, T lymphocytes, B lymphocytes or NK cells. These results also suggest that zinc-deficiency induces stress responses and the responses may have in part participated in increased actions of the number of granulocytes and monocytes during zinc-deficiency, and induce thymus atrophy and adrenal hypertrophy.

Key Words zinc-deficiency, white blood cells, granulocyte and lymphocytes, eosinophil and basophil

Zinc is known to play an important role in immune regulation, gene transcription and other fundamental physiological processes (1, 2). Zinc is also a cofactor for more than 300 enzymes such as alcohol dehydrogenase (ADH), superoxide dismutase (SOD), RNA polymerase, alkaline phosphatase and carbonic anhydrase (1). Among the zinc enzymes, there are oxidoreductases, transferases, hydrolases, lysases, isomerases and ligases. Therefore, general cell functions are influenced by the zinc concentration. About 60% of zinc is found in skeletal muscles and approximately 30% bound to bone, while plasma zinc concentration is only 12–16 μmol in humans (3–5). In the serum, zinc is predominantly bound to albumin (~60%), α2-macroglobulin (~30%) and transferrin (~10%) (3). Although the plasma zinc pool reflects only a minute portion of the total body zinc, this part is known to be a highly mobile and physiologically important pool (2, 3).

It is generally accepted that the recommended daily allowances of zinc are 2–3 mg/d for infants, 4–8 mg/d for children, 7–10 mg/d for teenagers and adults and 10 mg/d for pregnant women and lactating women (6–8). Zinc is found in a variety of foods, beans, nuts, whole grains and cereals, red meat, liver and seafood such as oyster and botargo (6, 9). On the other hand, most vegetables are not ready sources of zinc due to the presence of phytate, which chelates zinc and inhibits its absorption (2, 10).

According to the estimated prevalence of zinc-deficiency, it is widespread and affects the health and well being of populations worldwide (11). There are some groups that are at high risk of zinc-deficiency such as elderly people, vegetarians and patients with renal insufficiency (11, 12). In persons suffering from zinc-deficiency, clinical signs are depressed immunity,
impaired taste and smell, onset of night blindness, impairment of memory, and decreased spermatogenesis in males (3). Inadequate intake of zinc is accompanied by a number of clinical manifestations, which may prove fatal unless treated (10). Severe zinc-deficiency is characterized by severely depressed immune functions, frequent infections, bullous pustular dermatitis, diarrhea, alopecia, increased susceptibility to infections and mental disturbances (8). Even mild zinc-deficiency, which is widely spread in contrast to severe zinc-deficiency, depresses the immunity of humans (13).

It is well known that the functions of innate immunity are disturbed by zinc levels and the zinc-contents of granulocytes decreased by zinc-deficiency (2, 13). Innate immunity as the first line of defense represents a natural protection against infections. It is non-specific and responds to different antigens in the same way, indicating that it is not able to utilize memory cells. For example, natural killer (NK) cell activity, phagocytosis and generation of the oxidative burst by macrophage and neutrophil are impaired by decreased zinc levels (12). Not only the natural immune system but also the acquired immune system such as T cells function and B cell maturation are influenced by zinc-deficiency (2, 12).

On the other hand, the distribution of white blood cells is also essential for the immune responses, because the continuous migration of immune cells ensures the detection of antigens and neoplasms, and promotes cellular interactions that enable the immune system to execute rapid and effective responses (14). Many reports showed that the distribution of white blood cells in the circulating blood was changed by various stressful stimuli or circumstances such as body-restraint (15), simulated microgravity (16), exhaustive exercise (17, 18) and social stress (14). The distribution change in white blood cells and concentration of corticosteroids and catecholamines are considered the most regular and typical manifestations of stress (14, 15, 18). Furthermore, reactive oxygen species and cytokines also mediate immune cells. It is well known that proinflammatory cytokines such as interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)-α increase granulocytes (19, 20).

These findings suggest that zinc-deficiency stimulates the hypothalamus-pituitary-adrenal stress axis, leading to increased change in serum corticosterone and induced change in white blood cells (19). However, the effect of zinc-deficiency on the distribution of white blood cells (lymphocytes, monocytes, neutrophils, eosinophils and basophils) has not been systematically studied. The elucidation of the effects of zinc-deficiency on the distribution changes in white blood cells resulting from the altered responses of other physiological defense systems such as the autonomic nervous system and the endocrine system is crucial from the point of view of nutritional sciences and nutritional physiology. In the present study, therefore, the effects of zinc-deficiency on the number of white blood cells (total white blood cells (WBCs), neutrophils, eosinophils, basophils, monocytes and lymphocytes) in rats were studied. The effects of zinc-deficiency the number of lymphocyte subsets (T lymphocyte, B lymphocyte and NK cell), and on serum zinc, corticosterone, and IL-6 concentrations were also studied.

METHODS AND MATERIALS

Experimental procedure. The present study was carried out according to the experimental protocol shown in Fig. 1. During the experimental period, the rats were fed for 4 wk with the zinc deficient diet and the normal diet, and the number of white blood cells (neutrophil, eosinophil, basophil, lymphocytes and monocyte) were analyzed according to the protocol (Fig. 1). On the next day after the final day of the experiment, visceral organs (thymus, spleen and adrenals) were isolated and weighed, and then plasma samples were prepared. Plasma zinc, corticosterone and IL-6 concentrations

![Fig. 1. Experimental protocol used in the present study. After acclimation for 5 d, the zinc deficient diet (Zn=0.7 mg/kg diet) and the control diet (Zn=34.8 mg/kg diet) were provided to the ZDD group and CON group, respectively. Rats of the ZDD group and CON group were fed for 4 wk with the zinc deficient diet and the normal diet (Table 1). Down-arrow: Blood (the number of white blood cells, lymphocytes, monocytes, neutrophils, eosinophils and basophils, and the number of red blood cells, hematocrit, and hemoglobin concentration) analyses were carried out, and then visceral organs (thymus and adrenals) were isolated and weighed. Further, serum samples were prepared by our routine method, and the number of lymphocyte subsets (T lymphocytes, B lymphocytes and NK cells), serum Zn, corticosterone and IL-6 concentrations were assayed on the final day of the experimental period.](image-url)
Table 1. Composition of the experimental diet used in the present study.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Composition (g/kg diet)</th>
<th>CON</th>
<th>ZDD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg white</td>
<td>200.0</td>
<td>200.0</td>
<td></td>
</tr>
<tr>
<td>Dextrinized cornstarch</td>
<td>529.5</td>
<td>529.5</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Cellulose</td>
<td>50.0</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td>Soybean oil</td>
<td>70.0</td>
<td>70.0</td>
<td></td>
</tr>
<tr>
<td>Mineral mix (+zinc)</td>
<td>35.0</td>
<td>—</td>
<td>35.0</td>
</tr>
<tr>
<td>Mineral mix (−zinc)</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Vitamin mix (AIN-9 3G-VX)</td>
<td>10.0</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3.0</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>tert-Butylhydroquinone</td>
<td>0.014</td>
<td>0.014</td>
<td></td>
</tr>
</tbody>
</table>

CON: control group and ZDD: zinc deficient group.
1 Mineral mix used AIN-9 3G-MX.
2 ZnCO3 was omitted from AIN-9 3G-MX.

and the number of lymphocyte subsets (T lymphocyte, B lymphocyte and NK cell) were also determined.

Animal care. Three week old Sprague-Dawley male rats (CLEA Japan, Inc., Tokyo) were randomly divided into the zinc deficient diet (ZDD: n = 8, the initial body weight = 75.6 ± 1.4 g, mean ± SE) group and the control diet (CON: n = 8, the initial body weight = 75.3 ± 1.6 g, mean ± SE) group after the acclimation for 5 d. The rats of ZDD group were fed with the zinc deficient diet (0.7 mg zinc/kg diet) and the rats of CON group were fed with the normal requirement zinc diet (34.8 mg zinc/kg diet), the control diet (21). As shown in Table 1, rats of both groups received AIN-93G diet with egg-white being the protein source (Oriental Yeast Co., Ltd., Yokohama). The concentration of zinc in the diets of both groups was determined with an atomic absorption analysis. Because the anorexic eating patterns of the ZDD group were not synchronized, one-to-one pair feeding was conducted in the experiments (22). Care was taken to avoid zinc contamination by housing the rats in stainless steel wire-mesh cages, and by providing food in stainless-steel feeders and distilled water in plastic bottles with stainless-steel sipper tubes (23). The rats were maintained at a temperature of 23–25°C, a relative humidity of 50–60% and light was automatically provided from 7:00–19:00; they had access to food and water all the time (24, 25). The body weights of rats and food and water intake were determined at 9:00–10:00 a.m. each day (26, 27).

All experimental and animal care procedures were approved by the Committee on Animal Care and Use of Waseda University and followed the “Guiding Principle for the Care and Use of Animals in the Field of Physiological Sciences” of the Physiological Society of Japan (28). The experimental protocol was approved by the Animal Ethics Committee, Faculty of Human Sciences, Waseda University (No. 07J025, approved June 12, 2007). The experiment was performed with the least possible pain or discomfort to rats (24, 25).

Cell count analyses. According to the protocol presented in Fig. 1, the analyses of total WBCs, neutrophils, eosinophils, basophils, monocytes, lymphocytes, red blood cells, hemoglobin concentration and hematocrit were performed by a flow cytometry technique (29–31). Fifty microliters blood samples were collected with microcapillary tubes coated with EDTA-2K (Wako Pure Chemical Industries, Ltd., Osaka) from the tail vein at 9:00 a.m. (20). Blood samples were immediately diluted by 20% with CELLPACKTM (Sysmex Co, Hyogo). These blood samples were analysed using a hematology analyzer system (Model SF-3000 and SFVU-1, Sysmex Co) (32, 33). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated by the following equations (34).

MCV (fL) = hematocrit (%) × 10^2/(the number of red blood cells × 10^12/μL)
MCH (pg) = hemoglobin concentration (g/dL) × 10^2/(the number of red blood cells × 10^12/μL)
MCHC (%) = hemoglobin concentration (g/dL) × 10^2/hematocrit (%)

Sample collections. On the final day of the experiment, animals were anesthetized intraperitoneally with sodium pentobarbital (50 mg/kg per body weight). Blood samples were collected with vacuum tubes (Terumo, Tokyo) from the abdominal aorta, kept for 30 min at room temperature, and then centrifuged at 4°C and 3,000×g for 10 min to obtain serum. The collection was performed at 10:00–11:00 a.m. and quickly finished. Thymus, spleen and adrenals were rapidly isolated and weighed. Serum samples were stored at −80°C until further analyses.

Analyses of subsets of lymphocytes. The subsets (T lymphocyte, B lymphocyte and NK cell) of lymphocytes were determined by a direct immunofluorescent staining with a flow cytometric analysis (35). We used the Rat T/B/NK Cell Cocktail (Fluorescence-labeled antibody cocktail, Becton Dickinson, USA): APC-conjugated CD3 for T lymphocyte (clone 1F4); fluoresce in isothiocyanate (FITC)-conjugated anti-rat CD45RA for B lymphocyte (clone OX-33); and Phycoerythrin (PE)-conjugated anti-rat CD161a for NK cells (clone 10/78). One hundred microliters of blood was collected from the abdominal aorta and incubated with 5 μL of the appropriate primary antibodies for 30 min at room temperature in the dark. After that, 1 mL of lysing solution (BD FACSTM Lysing Solution, Becton Dickinson) was added, and after an incubation of 10 min, the samples were centrifuged at 1,500×g for 5 min at 4°C. The supernatant was discarded and the cells were resuspended with 1 mL of phosphate buffered saline (PBS) solution before being centrifuged again. After the second centrifugation (1,500×g for 5 min at 4°C), the supernatant was discarded and replaced with 0.5 mL of 1% paraformaldehyde in PBS to stabilize the cells. The cells were used for analysis of lymphocyte subset after the cells were filtered with a Cell Strainer (a strong nylon mesh with 40 micron pores, Falcon, BD FalconTM, USA). The flow
cytometer used in the present study is a system FACS Calibur (Becton Dickinson).

Assays of serum zinc, corticosterone and IL-6 concentrations. Serum zinc concentration was assayed spectrophotometrically. The assay was carried out with a Zn-test Wako kit (Wako Pure Chemical Industries, Ltd.). The microplate reader used for the zinc assay was a DTX 800 Multimode Detector (Beckman Coulter, Fullerton, CA). Serum corticosterone concentration was determined using an Enzyme-linked immunosorbent assay (ELISA) kit (Diagnostic Systems Laboratories, Texas). Serum IL-6 concentration was determined using an Enzyme-linked immunosorbent assay Wako kit (Wako Pure Chemical Industries, Ltd.). The microplate reader used for the corticosterone and IL-6 assay was a DTX 800 Multimode Detector (Beckman Coulter). The samples were analysed in duplicate and averaged for each measurement.

Statistics analyses. Experimental data were presented as mean±standard error (SE). The effects of zinc-deficiency on the body weight and the visceral organ weights, red blood cells, hemoglobin concentration, hematocrit, MCV, MCH and MCHC were tested by one-way ANOVA. The effects of zinc-deficiency on the number of white blood cells were evaluated by ANOVA for repeated measures. Subsequent post hoc analyses to determine significant differences between two groups were performed by Fisher’s protected least significant difference (PLSD) test. These analyses were carried out by the StatView 5.0 computer software (SAS Institute, Cary, NC). The differences were considered significant when p was <0.05.

RESULTS

Effects of zinc-deficiency on the body weight, body weight gain, total food intake, food efficiency, total water intake and serum zinc concentration

Figure 2 shows the changes of food intake (A), water intake (B) and body weight (C) during the experimental period. As shown in Fig. 2A, no significant changes in food intake in either group were observed during the experiment. However, as shown in Fig. 2B and C, the water intake and body weight in the ZDD group were gradually inhibited as the experimental period progressed, as compared with those of the CON group. The body weight, body weight gain, total food intake, food efficiency (fuel the ratio of the body weight gain to total food intake), total water intake and total zinc intake during zinc-deficiency are summarized in Table 2. The body weight of the ZDD group on the final day of the experiment was 0.25 times (p<0.001) lower than that of the CON group. Total food intake showed no significant difference between the two groups. However, the body weight gain, food efficiency, total water intake and total zinc intake of the ZDD group during the experimental period were 0.44 times (p<0.001), 0.45 times (p<0.001), 0.74 times (p<0.001) and 0.02 times (p<0.001) markedly lower than that of the CON group, respectively, suggesting that the ZDD group was in a state of severe zinc-deficiency. In fact, as shown in Fig. 3, zinc-deficiency induced a significant decrease in serum zinc concentration. The serum zinc concentration (0.31±0.08 µg/mL, mean±SE) of the ZDD group was 0.17 times (p<0.001) lower than that (1.80±0.25 µg/mL, mean±SE) of the CON group (Fig. 3).

Table 2. Effects of zinc-deficiency on the body weight, body weight gain, total food intake, food efficiency, total water intake, and total zinc intake during the experimental period.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>ZDD/CON</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>161±4</td>
<td>113±5***</td>
</tr>
<tr>
<td>Body weight gain (g)</td>
<td>85±3</td>
<td>38±4***</td>
</tr>
<tr>
<td>Total food intake (g)</td>
<td>274±6</td>
<td>271±6</td>
</tr>
<tr>
<td>Food efficiency1</td>
<td>0.28±0.01</td>
<td>0.13±0.02***</td>
</tr>
<tr>
<td>Total water intake (g)</td>
<td>568±19</td>
<td>418±33**</td>
</tr>
<tr>
<td>Total zinc intake (mg)</td>
<td>10.4±0.24</td>
<td>0.21±0.00***</td>
</tr>
</tbody>
</table>

Values: mean±SE (n=8/group). CON: control group and ZDD: zinc deficient group.
1Food efficiencies were calculated from the ratio of the body weight gain to total food intake.
Statistics: *p<0.05, **p<0.01 and ***p<0.001 (vs. the CON group, by one way ANOVA).
Effects of zinc-deficiency on the relative weight of visceral organs

The relative weight of the thymus per body weight of the ZDD group was 0.63 times \((p<0.01)\) lower than that of the CON group (Fig. 4A). The relative weight of spleen per body weight showed no significant difference between groups (Fig. 4B). On the other hand, the relative weight of adrenals per body weight of the ZDD group was 1.60 times \((p<0.001)\) higher than that of the CON group (Fig. 4C).

Effects of zinc-deficiency on serum corticosterone and IL-6 concentrations

Figure 5 shows the effects of zinc-deficiency on serum corticosterone and IL-6 concentrations. The serum corticosterone concentration \((1.14\pm0.11 \mu g/mL, \text{mean}\pm\text{SE})\) of the ZDD group was 1.48 times \((p<0.05)\) higher than that \((0.77\pm0.11 \mu g/mL, \text{mean}\pm\text{SE})\) of the CON group (Fig. 5A). However, no significant differences in serum IL-6 concentration \((\text{ZDD group}=22.7\pm5.5 \text{ pg/mL, mean}\pm\text{SE} \text{ and CON group}=31.8\pm4.5 \text{ pg/mL, mean}\pm\text{SE})\) between the two groups were observed (Fig. 5B).

Effects of zinc-deficiency on red blood cells, hemoglobin concentration, hematocrit, MCV, MCH and MCHC values

The number of red blood cells, hemoglobin concentration, hematocrit, MCV, MCH and MCHC values after the experimental period for both groups are shown in Table 3. There were no significant differences in these hematological parameters between the groups, suggesting that significant movement of water from extracellular to intracellular compartments during the experimental period can be neglected.

Effects of zinc-deficiency on the number of total WBCs, neutrophils, eosinophils, basophils, monocytes and lymphocytes

As shown in Fig. 6A, the number of total WBCs of the ZDD group in the 2nd, 3rd and 4th week was 1.04 times, 1.29 times \((p<0.05)\) and 1.63 times \((p<0.001)\) higher than that of the CON group, respectively (Fig. 6A). The number of neutrophils of the ZDD group in the 1st, 2nd, 3rd and 4th week was 1.30 times, 1.63 times \((p<0.05)\), 2.51 times \((p<0.001)\) and 4.58 times \((p<0.001)\) higher than that of the CON group, respectively (Fig. 6B). The number of eosinophils of the ZDD group in the 2nd, 3rd and 4th week was 1.36 times, 2.51 times \((p<0.001)\) and 5.04 times \((p<0.001)\) higher than that of the CON group, respectively (Fig. 6C).
Zinc-Deficiency Induced Changes of Rat WBCs

The effects of zinc-deficiency on the number of total white blood cells (WBCs), neutrophils, eosinophils, basophils, monocytes and lymphocytes (T lymphocytes, B lymphocytes and NK cells) in weaned rats during dietary zinc-deficiency for 4 wk. In the present study, therefore, the effects of zinc-deficiency on the number of white blood cells during the experimental periods were analyzed in weaned male rats. The effects of zinc-deficiency on visceral organ weights, serum zinc, corticosterone and IL-6 concentrations were also examined.

Zinc is essential to the function of DNA polymerase and RNA polymerase and the activity of the transcriptional regulator family, zinc finger DNA binding proteins (1–5). Their involvement in nucleic acid synthesis could explain the effects of zinc-deficiency on lymphoid cell proliferation of the thymus. It is well known that glucocorticoids are adrenal steroid hormones which induce the apoptosis of thymus and spleen cells (36–38). Martin et al. (39) reported that serum glucocorticoid concentrations were transiently increased by zinc-deficiency in rats. Continuous high levels of glucocorticoids resulting from zinc-deficiency cause apoptosis of lymphoid cells (20). Further, the weight of adrenals of the ZDD group was significantly higher than that of the CON group (Fig. 4C), suggesting that zinc-deficiency induces mild stress responses (2, 39). Thus, it is becoming evident that the thymus atrophy which accompanies zinc-deficiency is mainly due to an alteration in the production of lymphocytes (19). The primary function of the thymus is the maturation of bone marrow hematopoietic stem cell-derived precursor T lymphocytes (36). Further, zinc is necessary for the activity of T lymphocytes, B lymphocytes and natural killer (NK) cells.

Table 4 shows the effects of zinc-deficiency on the number of blood lymphocyte subsets. No significant differences between the groups in the populations of T lymphocytes, B lymphocytes or NK cells were observed on the final day of the experimental period.

**DISCUSSION**

The purpose of the present study was to elucidate the effects of zinc-deficiency on the number of total white blood cells (WBCs), neutrophils, eosinophils, basophils, monocytes and lymphocytes (T lymphocytes, B lymphocytes and NK cells) in weaned rats during dietary zinc-deficiency for 4 wk. In the present study, therefore, the effects of zinc-deficiency on the number of white blood cells during the experimental periods were analyzed in weaned male rats. The effects of zinc-deficiency on visceral organ weights, serum zinc, corticosterone and IL-6 concentrations were also examined.

2.67 times \((p<0.05)\) and 2.52 times \((p<0.001)\) higher than that of the CON group, respectively (Fig. 6C). The number of basophils of the ZDD group in the 1st, 2nd, 3rd and 4th week was 2.72 times, 2.06 times, 4.37 times \((p<0.01)\) and 6.89 times \((p<0.001)\) markedly higher than that of the CON group, respectively (Fig. 6D). The number of monocytes of the ZDD group in the 1st, 2nd, 3rd and 4th week was 1.41 times, 1.90 times, 2.03 times \((p<0.01)\) and 2.57 times \((p<0.001)\) significantly higher than that of the CON group, respectively (Fig. 6E). However, no significant differences between the groups in the number of lymphocytes during the experimental period were observed (Fig. 6F). From these results, the effects of zinc-deficiency on the relative ratio of the ZDD to the CON group were observed in the order of basophil/neutrophil/eosinophil/monocyte/total WBCs.

**Effects of zinc-deficiency on the number of T lymphocytes, B lymphocytes and NK cells**

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thymic hormone. Thymulin promotes T lymphocyte maturation and cytotoxicity production (40). Thymulin activity is dependent on plasma zinc concentrations so that changes in thymulin activity affect T lymphocyte maturation (40). In the present study, however, the number of peripheral T lymphocyte did not change (Table 4).

Paterson and Bettger (8) reported that dietary zinc-deficiency for 3 wk increased significantly the number of neutrophils and monocytes in weanling male rats. These results agree with our present data (Fig. 6B and E). On the other hand, there are no data regarding the response processes of the number of eosinophils or basophils during zinc-deficiency (Fig. 6C and D). It is known that basophils and eosinophils play an important role in allergic response or inflammation (2). Therefore, increased actions of basophils and eosinophils during zinc-deficiency may be associated specifically with some sort of inflammation. Interestingly, IL-6 levels were not changed by zinc-deficiency (2). In acute phase response, during zinc-deficiency, granulocytes and macrophages secrete a number of cytokines, IL-1, IL-6 and IL-8 into the circulation. The possible mechanisms may be closely connected with oxidative stress. In fact, zinc-deficiency reduce the expression of metallothionein, a free radical scavenger which may induce tissue damage (2, 39). Zinc could also be critical for some cytokine activity. For instance, it has been demonstrated that the production or the biological activity of IL-1, IL-4, IL-6, IFN-γ and TNF-α are affected by zinc-deficiency. In addition, zinc-deficiency in humans affects the cytokine production of TH1 cells leading to an imbalance among immune cells (40). Moreover, glucocorticoids enhance the release of neutrophils from bone marrow and act to inhibit apoptosis in neutrophils (39).

Inflammation actions and stress responses may play a part in the response of the number of granulocytes such as basophils, eosinophils and neutrophils during zinc-deficiency. Increased actions of the number of granulocytes may be at least partially physiologic, representing a shift in the ratio of freely circulating and marginal portions of the intravascular pool. The increase of circulatory granulocytes may also reflect a pathologic state such as infection or tissue necrosis, where the input of granulocytes into the intravascular compartment from the bone marrow storage pool increases (8). From these results and suggestions, although the mechanism of its action is unknown, an investigation of the kinetics of the production and compartment of numbers of granulocytes during the zinc-deficiency will be necessary (40).

In conclusion, during zinc-deficiency granulocytes (=neutrophils, eosinophils and basophils) and monocytes are mobilized from the marginal pool or bone marrow into the circulation pool without changing the number of lymphocytes (41). These results suggest that zinc-deficiency induces a dramatic redistribution of white blood cells in the circulation without changing the number of lymphocyte subsets (T lymphocyte, B lymphocyte and NK cell) and these responses of white blood cells during the experimental period of zinc-deficiency are at least sustained for 4 wk. Further studies are needed to clarify the mechanism of zinc-deficiency-induced changes in the number of granulocytes and monocytes.

Acknowledgments
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