



Single nutrient deficiency and cell-mediated immune responses

I. Zinc¹⁻⁴

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ABSTRACT The thymus of rats provided zinc-deficient diet weighed less than the thymus of animals fed zinc-containing control diet. The antibody-forming cell response in the spleen was reduced. Cytotoxic response of spleen cells of zinc-deficient mice immunized in vivo was decreased whereas after sensitization in vitro the response was comparable to that seen in zinc-replete animals. Natural killer cell activity and antibody-dependent cell-mediated cytotoxicity were increased, particularly the former. These observations suggest that dietary zinc intake is an important factor modulating cell-mediated immune responses. *Am. J. Clin. Nutr.* 33: 736-738, 1980

There is growing recognition of the critical role of dietary intake and nutritional status in modulating immune responses and susceptibility to infection (1, 2). In children with moderate-severe protein-energy malnutrition, there is a significant depression of cell-mediated immunity. Similar observations have been made in individuals with predominant deficiencies of iron (3-6), folate (7), and zinc (6; R. K. Chandra, manuscript in preparation). However, in man it is difficult to evaluate the effect of deficiency of a single nutrient on various parameters of the immune response. We have studied a broad range of cell-mediated immune responses in zinc-deprived rats and mice. Similar data on animals selectively deprived of vitamin A or pyridoxine or protein will be reported separately (8, 9; R. K. Chandra, manuscript in preparation).

Materials and methods

Groups of 3-week-old Sprague-Dawley rats bred in our animal house were fed either on a zinc-deficient diet containing approximately 6 ppm zinc or a control diet containing approximately 55 ppm zinc. The two groups of rats were pair fed to exclude secondary deficiencies associated with reduced intake. Triple-distilled water was provided ad libitum.

Groups of C57B1/6J mice were similarly provided with either zinc-deficient or control diet.

Serum zinc concentration was determined directly in an atomic absorption spectrophotometer. Hair zinc content was estimated by ashing followed by atomic absorption spectrophotometry.

After 4 to 5 weeks on either the zinc-deficient or zinc-replete diet, some rats were killed to determine the weight of the thymus and the spleen. In mice, natural killer (NK) cell activity was estimated by a direct micro-cytotoxicity ⁵¹Cr release method (10). Antibody-dependent cell-mediated cytotoxicity of spleen cells was determined using ⁵¹Cr-labeled chicken erythrocytes as targets and effector/target ratio of 50:1 (11). Some rats were immunized intraperitoneally with sheep red blood cells and mice were injected with EL-4 lymphoma tumor cells and killed several days later to estimate the number of antibody hemolytic plaque-forming cells in the spleen using Jerne's technique (12) or the cytotoxic activity of

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spleen cells in a microplate ^{51}Cr -release assay (13), respectively.

Statistical analysis used Student's *t* test and paired *t* test.

Results and discussion

Zinc-deficient rats had a slower growth and their thymus weighed less (Table 1). The weight of the spleen was comparable in zinc-deficient and control rats. PFC response was significantly lower in zinc-deprived animals. This response is dependent upon the thymus and these findings point to thymic atrophy and hypofunction associated with zinc deficiency. A decrease in the number of helper T-cells or of precursors of antibody forming cells or increased suppressor activity may underlie such alterations in immune responses. Our data are compatible with similar observations recently reported from other centers (14–16).

There was a significant increase in NK cell activity and a slight increase in ADCC in zinc-deprived mice (Fig. 1). This dissociation between alterations in NK cell function and antibody-dependent cell-mediated cytotoxicity points to the possibility of a differential effect of zinc deficiency on either two subsets of lymphocytes mediating these two activities or on the two functions subserved by the same cell. It has been suggested that cells bearing Fc receptors mediate both these functions. Our results are at slight variance from those of Fernandes et al. (15) who observed low NK cell activity although antibody-dependent cell-mediated cytotoxicity was normal.

The generation of cytotoxicity in spleen cells of zinc-deficient mice immunized in vivo was distinctly lower at two different effector/target cell ratios than that in control animals (Fig. 2). However, the cytotoxic response of

splenic lymphocytes against allogeneic cells after in vitro sensitization was comparable in the two groups. These data suggest that a zinc-deficient microenvironment impairs lymphocyte effector function. Zinc content of culture medium used in the in vitro immunization may have been adequate to permit antigenic stimulation whereas in vivo this could not occur.

The critical role of zinc in the activity of several metalloenzymes and metabolic pathways including DNA synthesis is established. Recent studies in children with acrodermatitis enteropathica suggest that low plasma zinc concentration is associated with impaired cell-mediated immunity and that zinc therapy corrects the immunologic deficit (R. K. Chandra, manuscript in preparation). Our data obtained on zinc-deficient mice and rats confirm that zinc-deprivation results in a significant alteration in cell-mediated immune responses. These observations are pertinent to

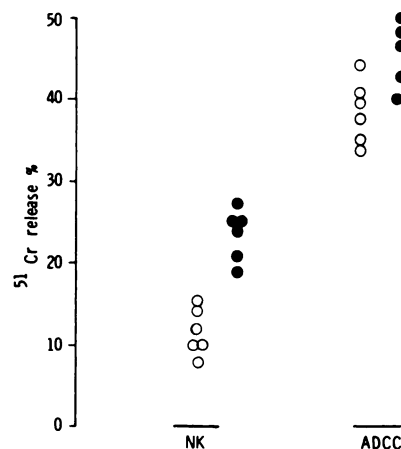


FIG. 1. NK cell activity and antibody-dependent cell-mediated cytotoxicity (ADCC) in zinc-deficient (●) and control (○) animals.

TABLE 1
Organ weights and direct plaque forming cell (PFC) response in rats

Group	No. of animals	Body weight	Thymus weight	Spleen weight	PFC per spleen
		g		mg	$\times 10^3$
Zinc deficient ^a	8	198 \pm 23 ^b	209 \pm 37	340 \pm 63	24.1 \pm 5.2
Zinc replete ^c	7	226 \pm 17	288 \pm 43	361 \pm 54	39.4 \pm 6.7
<i>P</i>		NS ^d	<0.05	NS	<0.01

^a Zinc concentrations in serum and hair were 31.2 \pm 11.1 $\mu\text{g}/100\text{ ml}$ and 123 \pm 15 ppm, respectively. ^b Values are shown as mean \pm SD. ^c Zinc concentrations in serum and hair were 118.7 \pm 14.3 $\mu\text{g}/100\text{ ml}$ and 219 \pm 22 ppm, respectively. ^d Not significant.

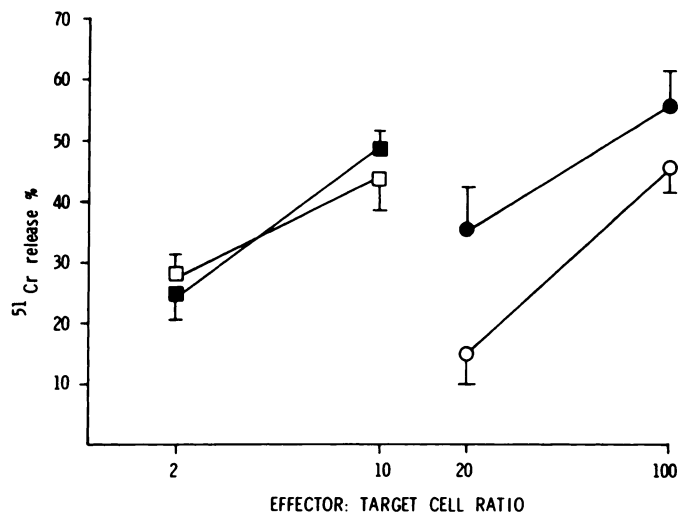



FIG. 2. Cytotoxicity response of zinc-deficient mice (open symbols) and controls (closed symbols) to immunization in vitro at effector/target cell ratios of 2:1 and

10:1, or to sensitization in vivo at effector/target cell ratios of 20:1 and 100:1. Data are based on observations in six to eight animals and are shown as mean \pm SE.

the clinical syndromes wherein zinc deficiency is frequently encountered, viz. protein-calorie malnutrition, intravenous feeding, acrodermatitis enteropathica, obesity, intestinal by-pass surgery, and others. 

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