

Effect of experimental zinc deficiency and repletion on some immunological variables in guinea-pigs

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1. Cellular and humoral immune responses were studied in guinea-pigs fed on zinc-deficient (ZnD), Zn-adequate (control) and Zn-replete diets containing 1.25, 50 and 100 mg Zn/kg diet respectively.
2. It was found that the ZnD guinea-pigs had significantly decreased ability to elicit delayed-type hypersensitivity (DTH) response against sheep erythrocytes as compared with controls on the 9th day of immunization. This was further substantiated by histological examination of DTH-positive skin sections.
3. A significant reduction in direct splenic plaque-forming-cell response and haemagglutinating-antibody titre was also observed in ZnD guinea-pigs.
4. Serum electrophoretic studies revealed a highly disordered protein profile with a significantly depressed value for γ -globulin.
5. Zn repletion of the previously ZnD group resulted in marked, though incomplete, restoration of immunological responses.

Zinc plays an indispensable role in the development and maintenance of immunocompetence and a number of other body-functions. Natural cases of its deficiency in man and domestic animals have been reported throughout the world. Human patients with low levels of serum Zn, and cattle having heritable disorders of Zn metabolism (Gershwin *et al.* 1985), had increased susceptibility to a variety of infectious disorders and defective cell-mediated immunity. There is little published work on immunological studies of experimental Zn deficiency in laboratory animals, particularly in guinea-pigs in which cell-mediated allergic reactions are readily induced and evaluated (Parker & Turk, 1974). The only report (Carlomagno *et al.* 1985) traced in the literature on immunological observations in experimental Zn-deficient (ZnD) guinea-pigs revealed that Zn deficiency suppressed the delayed-type hypersensitivity (DTH) response. The present work was undertaken, therefore, to study the effect of experimental Zn deficiency and repletion on certain cellular and humoral immune responses in guinea-pigs.

MATERIALS AND METHODS

Animals and diets

Recently weaned albino guinea-pigs (21-d-old) of an inbred colony of the English breed, weighing about 200-250 g, were obtained from the Disease-Free Small Animal House, Haryana Agricultural University, Hisar. These were fed *ad lib.* on a purified diet, the details of which have already been described (Gupta *et al.* 1985). All animals were kept individually in plastic cages and provided with double-distilled water *ad lib.* To avoid coprophagy, perforated acrylic sheets were fixed at the bottom of the cages.

Experimental design

The guinea-pigs were randomly divided into two groups containing twelve and six animals respectively. Animals of the first group were fed on a ZnD diet containing 1.25 (SE 0.12) mg Zn/kg for 45 d, and then divided into two subgroups of six animals each. The animals

of subgroup 1 continued to receive the ZnD diet while animals of subgroup 2 were given a Zn-replete (ZnR) diet (100 mg Zn/kg diet). All animals of the second main group were fed on a control Zn-adequate diet (50 mg Zn/kg diet) throughout the experiment.

All guinea-pigs were closely observed daily for clinical signs.

Serum Zn analysis

Blood was collected from the heart at the start of the experiment and then at days 45 and 60 of the experiment in sterilized tubes for serum separation. Serum Zn concentration was estimated using atomic absorption spectrophotometry (Horwitz, 1965).

Antigen

Sheep erythrocytes (SRBC) were used as the antigen in these studies. Fresh blood from a healthy sheep was collected in Alsever's solution. Before use, this was washed three times in 0.01 M-sodium-phosphate-buffered saline (9 g sodium chloride/l: PBS), pH 7.2.

Immunization

All guinea-pigs of different experimental groups were inoculated subcutaneously with 2×10^8 SRBC (suspended in PBS) in the hind-foot pad on day 60 of the experiment.

DTH response

SRBC-specific DTH reaction was elicited in guinea-pigs by injecting 25 μ l SRBC (5×10^7) in PBS (100 ml/l) into the dorsal side of the right ear of each animal on the 8th day of immunization. An equal amount (25 μ l) of PBS was injected into the dorsal side of the left ear, which served as control. DTH reaction was determined by measuring the specific increase in ear thickness 24 h after injection of the eliciting antigen as described by Tamura *et al.* (1973). For histological studies of DTH reaction, a piece of skin from the right and left ears was collected into buffered formal-saline solution (100 ml/l). Paraffin sections, 4–5 μ m thick, were cut and stained with haematoxylin and eosin.

Direct splenic plaque-forming cell (PFC) count

Guinea-pigs of different groups were killed on the 69th day of the experiment and their spleens were removed aseptically. The total number of direct-PFC per million spleen cells was determined by the Jerne Plaque assay (Jerne, 1974).

Haemagglutinating-antibody (HA) titre

HA titres were determined by the microtitre method (Tamura *et al.* 1973) in the sera of all guinea-pigs immediately after measuring DTH reaction.

Serum proteins

Total serum protein concentration was determined by the Biuret method (Wootton, 1974) and its fractions by paper electrophoresis (Huisman, 1963) on the day of immunization and thereafter on the 9th day of immunization.

Statistical analysis

The statistical significance was assessed by Student's 't' test.

RESULTS

Zn deficiency was successfully produced experimentally within 45 d in the guinea-pigs given the ZnD diet as evidenced by clinical signs and a reduction in serum Zn level. Food intake

among different experimental groups of guinea-pigs was almost identical throughout the experiment.

DTH response

The average increase in skin thickness and degree of cellular reactions in different experimental groups are given in Table 1. It was observed that the mean increase in skin thickness due to SRBC-specific DTH was significantly ($P < 0.01$) lower in the ZnD group as compared with the control. Although an increased skin thickness in the ZnR group was also noticed, the difference from the control value was not significant.

A swelling in the form of induration and nodulation appeared at the site of injection in the right ear but no such swelling was perceptible in the left ear (control). Histological examination of the indurated area of skin from guinea-pigs given a control Zn-adequate diet revealed oedema and intense infiltration of lymphocytes, macrophages and neutrophils in the dermis (Plate 1 (a)). The cellular infiltration was mainly around the blood vessels. In the ZnD group, the oedema and cellular infiltration was of a very-mild degree (Plate 1 (b)) while in the ZnR group it was almost of the same intensity as that of the control group. No oedema or cellular infiltration was noticed in sections from the left (control) ear.

PFC and haemagglutination assay

The mean direct-PFC per million spleen cells and HA titres for each experimental group are given in Table 2. It is apparent that the mean PFC per million spleen cells and HA titres were significantly ($P < 0.01$) lower in the ZnD group compared with the control group. The ZnR group also showed lower values, in comparison with those of the controls, but the differences were not significant.

Serum proteins

Mean values for total serum protein and the different serum protein fractions for each experimental group are shown in Table 3. Total serum protein concentration in the ZnD group was significantly ($P < 0.05$) lower than that in the control group on the 60th day of the experiment, at which time the guinea-pigs were immunized to SRBC antigen. Electrophoretic evaluation of serum from the ZnD group also revealed a highly disordered distribution of the protein profile with significantly ($P < 0.01$) decreased levels of γ -globulin and albumin as compared with the controls. The value for γ -globulin in the ZnD group 9 d post-immunization was almost the same as that on the day of immunization, but in the control group there was a considerable increase in the γ -globulin level. In the ZnR group, the γ -globulin level was slightly increased but the difference when compared with the control group was not significant.

DISCUSSION

Anorexia is generally accepted as inevitable during Zn deficiency and pair-feeding with a Zn-adequate control diet is considered to be necessary. However, in the present study food intake by the animals in different groups was almost identical. McBean *et al.* (1972) and Gupta *et al.* (1985) also did not notice any effect of Zn deficiency on food intake in guinea-pigs. Thus the results reported in the present study are of pure Zn deficiency unaccompanied by reduced food intake.

The results of the cell-mediated immune (CMI) response to SRBC in the present study are in accordance with the findings of Fraker *et al.* (1982) in mice and Carlomagno *et al.* (1985) in guinea-pigs. They reported impairment of DTH responses to dinitro-fluorobenzene and tuberculin respectively in ZnD animals. Cellular reactions of DTH observed in the present study are almost similar to that reported by Mitsuoka *et al.* (1978) in SRBC-immunized mice. The DTH responses are complex and involve interplay between

Table 1. *Delayed-type hypersensitivity response in zinc-deficient, Zn-repleted and control guinea-pigs immunized with sheep erythrocytes†*
(Mean values with their standard errors for six animals/group)

Group	Specific increase in skin thickness (mm)		Histological cellular reaction
	Mean	SE	
Control	0.234	0.003	+++
Zn-deficient	0.144**	0.002	+
Zn-repleted‡	0.204	0.023	++

+++ , Intense; ++ , moderate; + , mild.

Mean values were significantly different from those of the control group: ** $P < 0.01$.

† For details of diets and immunization procedures, see pp. 149–150.

‡ Repleted on 45th day of depletion.

Table 2. *Antibody-mediated immune responses in zinc-deficient, Zn-repleted and control guinea-pigs immunized with sheep erythrocytes†*
(Mean values with their standard errors for six animals/group)

Group	Direct PFC/10 ⁶ spleen cells		HA titre	
	Mean	SE	Mean	SE
Control	540.00	67.08	6.40	0.88
Zn-deficient	140.00**	27.38	2.00**	0.57
Zn-repleted‡	460.00	120.41	5.60	0.88

PFC, plaque-forming cells; HA, haemagglutinating antibody.

Mean values were significantly different from those for the control group: ** $P < 0.01$.

† For details of diets and immunization procedures, see pp. 149–150.

‡ Repleted on 45th day of depletion.

the T_D sub-population of T-lymphocytes (T-cells) and secreted mediators as well as macrophages, the detailed mechanism of which is not yet completely understood. Lyt-1 T helper cells, which are closely related to T_D lymphocytes (Askenase, 1980), have shown altered functionality both in vivo against antigen and in vitro against concanavalin A (DePasquale-Jardieu & Fraker, 1981) during Zn deficiency in mice. Since a reduction in in vivo performance of T-cells involved in CMI response has been reported in ZnD animals (Fernandes *et al.* 1979; Chandra & Au, 1980), it may be inferred that at least part of the reduction in DTH in Zn deficiency is due to a reduction in T_D effector functions.

Splenic PFC counts have been reported only as an index of humoral immune stimulation against SRBC in Zn-deficient animals (Fernandes *et al.* 1979; Beach *et al.* 1980) without concomitant measurement of antibody titre response which is one of the valid functional indices of humoral immunity. In the present study, however, a significant reduction in HA titre was observed in ZnD guinea-pigs together with a marked reduction in direct PFC response to SRBC compared with the controls. These results indicate that Zn deficiency *per se* causes extensive impairment of T-helper cell functions that are required for optimal

Table 3. Total serum protein and serum protein fractions (g/l) in zinc-deficient, Zn-repleted and control guinea-pigs at 0 and 9th days of immunization with sheep erythrocytes† (Mean values with their standard errors for six animals/group)

Day of immunization	0		9	
	Mean	SE	Mean	SE
Zn-deficient				
Total protein	54*	3.1	56*	0.2
Albumin	32**	0.3	33**	0.7
α -Globulin	12	0.4	13	0.3
β -Globulin	7	0.3	7	0.2
γ -Globulin	1.5**	0.3	2**	0.1
Zn-repleted‡				
Total protein	77	5.2	78	2.8
Albumin	50	1.5	50	0.6
α -Globulin	16	0.9	17	0.6
β -Globulin	8	1.0	7	0.5
γ -Globulin	4	1.2	5	0.5
Control				
Total protein	75	7.3	80	2.9
Albumin	48	0.8	49	0.3
α -Globulin	14	1.0	16	0.5
β -Globulin	6	0.6	7	0.1
γ -Globulin	6	0.5	10	2.3

Mean values were significantly different from those of the control group: * $P < 0.05$, ** $P < 0.01$.

† For details of diets and immunization procedures, see pp. 149–150.

‡ Repleted on 45th day of depletion.

antibody-mediated response (Borum, 1972) against thymus-dependent antigen (SRBC). The studies of Fraker *et al.* (1977) have clearly demonstrated that the impairment is primarily attributable to T-helper cells and may have little effect on B lymphocytes.

Limited information appears to be available in the literature regarding the serum protein profile of immunized ZnD animals. Beach *et al.* (1980) observed a high variation in the serum immunoglobulin profile without any significant change in the total γ -globulin level on the 4th day of immunization to SRBC in ZnD mice. In the present study, however, the γ -globulin concentration in the ZnD group was significantly ($P < 0.01$) lower than that of the controls on the 9th day of immunization. These results also suggest that Zn deficiency does have an adverse effect on antibody-mediated immune response. Impairment of cellular and humoral immune responses due to Zn deficiency may be due partly to decreased activity of DNA polymerase, RNA polymerase and thymidin kinase (Kirchgessner *et al.* 1976) which are required for rapid proliferation of immunocytes and protein synthesis.

The findings for the ZnR group showed that there was marked, though incomplete, restoration of immunological response. Very similar observations for Zn repletion have been made in mice (Fraker *et al.* 1978, 1982). However, it may be necessary to examine whether the impairment of immunological functions observed as a result of dietary Zn deficiency is completely reversible following repletion or whether there remain certain irreversible features of this condition, since repletion studies were carried out for 15 d only.

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EXPLANATION OF PLATE

Plate 1. Oedema and intense mononuclear infiltration in delayed-type hypersensitivity positive skin section (ear) of a guinea-pig given (a) a control, zinc-adequate diet and (b) a Zn-deficient diet (for details see pp. 149–150). Haematoxylin and eosin stain; magnification $\times 100$.

