British Journal of Nutrition (1986), **55**, 59–69

T-lymphocyte subsets and interleukin-2 production in zinc-deficient rats

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(*Received 11 March 1985 – Accepted 9 August 1985*)

1. It has been suggested that zinc-deficiency impairs cellular (T-lymphocyte-mediated) immune responses via a selective effect on helper T-lymphocytes. We have addressed this question in the rat by employing recently developed reagents in the form of monoclonal antibodies which specifically identify rat T-lymphocyte subsets (identifying total T-cells, helper T-cells and suppressor T-cells) and also by quantifying helper T-cell function by measurement of the helper T-cell-derived molecule interleukin-2 (IL-2).

2. Zn-deficiency induced T-cell atrophy (assessed morphologically and phenotypically with anti-rat T-cell monoclonal antibodies) in both peripheral blood and spleen. The use of these specific monoclonal antibodies failed to demonstrate a selective effect of Zn deficiency on the helper T-cell fraction of the total T-lymphocyte population.

3. In contrast, the results of functional assays of the T-lymphocyte response were dependent on the conditions of culture but suggested that the generation of IL-2 and its corresponding receptor were determined by the intracellular Zn status. Thus, in vivo, helper T-lymphocyte numbers are non-specifically reduced since other T-cell subsets are also reduced in response to appropriate stimulation. The functional consequences of this are dependent on the intracellular concentration of Zn but appear to influence both IL-2 production and its receptors on activated T-cells.

The effects of zinc-deficiency on the lymphoid system are multiple (Bach, 1981) but it has been suggested that the T-lymphocyte system is more susceptible to a deficiency in this trace element than other compartments of the immunological response. Thus, in the Zn-deficient animal, thymic atrophy, lymphopenia and impaired anti-tumour immunity and antibody production in response to heterologous erythrocytes have been reported (Fraker *et al.* 1978; Fernandes *et al.* 1979; Frost *et al.* 1981). Conversely, Fraker & Leucke (1981) failed to detect any effect of Zn deficiency on the mitogenic response of rodent spenocytes. In addition, the wide variation in lymphocyte responses between individual Zn-deficient rats reported by Gross *et al.* (1979*a*) does not fully support their conclusion that one of the fundamental defects induced by Zn-deficiency is that of impaired helper T-lymphocyte function. In recent years there have been a number of advances in immunological techniques which have permitted the fine dissection of the immune response at the cellular and molecular levels. Foremost among these has been the development of monoclonal antibodies (McAB) produced following the fusion of a single antibody-producing cell with an immortal malignant myeloma cell, the resultant 'hybridoma' manufacturing large quantities of a single antibody with a single specificity (Kohler & Milstein, 1975). Such McAB have become powerful tools in medicine and biology because of their specificity for a single epitope on a molecule present on a cell surface. Thus, for example, it is now possible to quantify functionally distinct T-lymphocyte sub-populations at the single cell level in the rat, mouse and human systems using specific McAB which bind to different molecules on the surfaces of helper T-cells and suppressor T-cells. A second important advance has been the recognition that many immunoreactive cell functions are mediated and regulated by soluble cytokines released locally at the site of the immune response. One such cytokine, a lymphokine formerly known as T-cell growth factor but now termed interleukin-2 (IL-2), is now recognized to play a central and dominant role in the generation of T-lymphocyte

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https://doi.org/10.1079/BJN19860010 Published online by Cambridge University Press

responses (Gillis, 1983). It is released by helper T-cells on appropriate mitogenic or antigenic stimulation. Receptors for IL-2 are present on activated, but not resting, T-lymphocytes and the binding of IL-2 by such cells results in continued proliferation of the activated T-cells until the IL-2 is depleted.

Because of our own finding of an association between impaired T-lymphocyte function in malnourished hospital in-patients (P. Dowd, J. Kelleher, B. E. Walker and P. J. Guillou, unpublished results), we wished to examine certain immunological aspects of Zn deficiency in an experimental model in which the added influence of protein–energy malnutrition, often seen clinically in association with Zn deficiency, was minimized. The present studies were designed to examine the effects of Zn deficiency on the numerical distribution of T-cell subsets in rat splenocytes using a battery of specific McAB. In addition we have examined the functional influence which Zn deficiency has on IL-2 production in vitro when the experiments are conducted in the presence of serum with normal and low Zn concentrations.

MATERIALS AND METHODS

Animals

Wistar rats initially weighing 100 g were used throughout these experiments. All rats were housed individually in stainless steel metabolism cages and given distilled, de-ionized water in Zn-free containers. To obtain pools of normal and Zn-deficient serum, two groups of ten rats per group were given either a purified diet with a normal Zn content or a Zn-deficient diet *ad lib.* for 4 weeks.

For all animals the diet was a semi-synthetic one based on dried albumin, sucrose and arachis oil with added vitamins and minerals sufficient to fulfil the requirements of the rat as laid down by the (US) National Academy of Sciences (1972). Zinc sulphate was omitted from the Zn-deficient diet which thus contained, by analysis, less than 1 mg Zn/kg. Zinc sulphate was added to the control diet to provide a Zn content of 40 mg/kg.

Blood from the rats was obtained by cardiac puncture into acid-washed glass tubes and the serum removed. The Zn content of each serum was measured by atomic absorption spectrophotometry and sera with low Zn concentrations were pooled, as were the sera with a normal Zn content, and stored at $-40°$ for further experiments. Splenocytes from the normal rats also served to provide a standard IL-2 preparation as detailed later (p. 62). Subsequent studies were performed on two further groups of eight rats. One group received the Zn-deficient diet and the remaining group were pair-fed on a group basis with the Zn-supplemented diet. Thus the mean daily dietary intake for the previous 24 h period was measured each day for the Zn-deficient rats and this quantity of Zn-supplemented diet was then given to each rat in the control group for the current 24 h period. After 4 weeks the rats were killed. Blood was removed by cardiac puncture and the spleen removed and weighed.

Peripheral blood cell studies

Peripheral blood obtained by cardiac puncture was heparinized and a total leucocyte count was performed. One drop of blood was used to make a smear for differential counting after staining with May–Grunwald–Giemsa haematological stain. The percentages and total Neutrophil, lymphocyte and monocyte counts were calculated for each animal.

The peripheral mononuclear cells were separated from the remaining whole blood after dilution with Hanks balanced salt solution (HBSS) and Ficoll-hypaque centrifugation. These were then treated with McAB for the quantification of rat T-cell subsets as detailed later (p. 61).

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Spleen cell studies

Spleen cells were isolated by mincing and teasing the spleen through a sterile copper mesh into HBSS. The debris was allowed to settle and was removed. Erythrocytes were lysed by hypotonic shock and the splenocytes suspended in RPMI 1640 (Flow Laboratories, Irvin, Scotland) containing 50 ml Zn-deficient rat serum/l and the percentage of viable cells counted. This suspension was then incubated for 2 h at 37° on plastic petri-dishes to remove adherent cells. At the end of this period, the non-adherent cells were removed and the plates washed with cold medium and again counted. A portion of this suspension was taken for incubation with McAB whereas the remainder was stimulated with Concanavalin-A as detailed below. For each spleen the total number of splenocytes, percentage adherent cells and number of non-adherent cells obtained per spleen were calculated.

Mitogenic stimulation

Non-adherent splenocytes (2×10^5) were cultured (four replicates) in 0·2 ml culture medium supplemented with 100 ml Zn-deficient or normal pooled rat serum/l. Unstimulated control cultures contained no mitogen. To stimulated cultures was added 5 μg Concanavalin-A (Con-A; Sigma Chemical Co., Poole, Dorset)/ml, this concentration having been previously determined as giving optimal responses in both normal and Zn-deficient rats. The culture medium consisted of RPMI 1640 containing gentamicin (Kirby–Warrick Pharmaceuticals Ltd) (160 μg/ml) and 2-mercaptoethanol (50 μmol/ml). Cultures were performed in ninety-six well-microplates (Nunc) incubated at 37° in a humidified atmosphere containing 50 ml carbon dioxide/l. After 24 h in culture, 100 μl supernatant fraction were removed from each well without disturbing the cell layer and stored at −70° for subsequent IL-2 measurement as described later (p. 62). This medium was replaced either with medium containing 100 ml Zn-deficient serum/l or medium containing normal rat serum as appropriate. Culture was then continued for a further 24 h. At 18 h before the termination of the cultures these were pulsed with [^{3}H]thymidine (0·1 μCi/well). Cultures were harvested on an automatic cell harvesting device and the thymidine incorporation measured in a β-counter. Raw counts/min values obtained in such assays were expressed as a stimulation index (SI) which is:

$$\text{SI} = \frac{[^3\text{H}]\text{thymidine uptake in Con-A-stimulated cultures (counts/min)}}{[^3\text{H}]\text{thymidine uptake in unstimulated cultures (counts/min)}}$$

Thus, for each animal studied two SI were obtained, one for splenocytes cultured in normal rat serum and the other for the same cells when cultured in Zn-deficient serum.

Measurement of T-cell subsets

T-cell subsets in peripheral blood and spleen were quantified by an indirect immunofluorescent technique as previously described (Guillou *et al.* 1984) using three monoclonal anti-rat T-cell antibodies. W3/13 is bound by thymocytes, most T-cells, plasma cells, polymorphs and stem cells. W3/25 antigen is expressed on most rat thymocytes and peripheral T-lymphocytes including cells of the helper phenotype. The Ox8 McAB labels all remaining peripheral T-cells which are unlabelled by W3/25, including suppressor-cytotoxic cells (Williams *et al.* 1977; Cantrell *et al.* 1982). These antibodies were obtained as ascitic fluid from Seralab and used at a concentration of 10 μg/ml. Each mononuclear cell suspension was incubated for 1 h at 4° with ascitic fluid, washed twice with phosphate-buffered saline (9 g sodium chloride/l) and incubated for 1 h with FITC-conjugated goat anti-mouse IgG (Seralab). After washing three times, the percentage of fluorescing cells was counted on a Leitz microscope fitted with epi-illumination.

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Table 1. *Differential effects of zinc deficiency on rat peripheral blood total leucocyte count, neutrophils and T-lymphocyte subsets binding monoclonal antibodies*

(Mean values and standard deviations for eight rats per group)

	Zn deficient		Pair-fed		Statistical significance of difference: P
	Mean	SD	Mean	SD	
Total leucocyte count ($\times 10^9$/l)	0·9	0·8	2·9	2·3	$< 0·05$
Neutrophils (%)	50	15	9	2	$< 0·01$
Lymphocytes (%)	48	14	88	4	$< 0·01$
Monocytes (%)	1·9	0·8	1·4	0·9	NS
Neutrophils ($\times 10^9$/l)	0·38	0·22	0·25	0·17	NS
Lymphocytes ($\times 10^9$/l)	0·53	0·70	2·57	2·05	$< 0·01$
Monocytes ($\times 10^9$/l)	0·02	0·02	0·04	0·05	NS
W3/13-positive cells (total T-cells) (%)*	83·5	7·4	79·1	8·0	NS
W3/25-positive cells (helper-inducer T-cells) (%)*	55·6	9·4	52·6	9·7	NS
Ox8-positive cells (cytotoxic-suppressor T-cells) (%)*	25·8	4·4	31·5	5·9	NS
W3/13-positive cells ($\times 10^9$/l)†	0·43	0·53	2·09	1·68	$< 0·01$
W3/25-positive cells ($\times 10^9$/l)†	0·30	0·39	1·43	1·24	$< 0·01$
Ox8-positive cells ($\times 10^9$/l)†	0·13	0·17	0·76	0·54	$< 0·01$

NS, not significant.

* Percentage of Ficoll-hypaque-separated peripheral blood mononuclear cells (i.e. lymphocytes) binding each monoclonal antibody.

† Numbers of antibody-binding cells calculated from the percentage peripheral blood cells binding each antibody and total number of lymphocytes ($\times 10^9$/l) on May–Grunwald–Giemsa stained smears for each individual rat.

IL-2 assays

The IL-2 content of 24 h Con-A-stimulated rat splenocytes was measured by the technique of Gillis *et al.* (1978) using the dose-dependent growth of an IL-2 dependent cloned murine cytotoxic T-cell line (CTLL-2). Duplicate portions of $\log_2$ dilutions of the supernatant fraction in question were placed in wells of a ninety-six-well, flat-bottomed microculture plate (Nunc). To each well was added 0·1 ml of a suspension of CTLL-2 cells at a cell density of 10^4/ml in RPMI 1640 containing 2-mercaptoethanol (50 μmol/ml), gentamicin (160 μg/ml) and 100 ml heat-inactivated fetal calf serum (FCS)/l. After 24 h incubation, 0·1 μCi [^{3}H]thymidine was added to each well and the microcultures harvested 6 h later. The amount of [^{3}H]thymidine incorporated into the cells was measured by scintillation counting. The concentration of IL-2 present in the supernatant fraction was calculated by the log-probit technique of Gillis *et al.* (1978) by comparison with a standard rat IL-2 preparation prepared in bulk cultures of Con-A-stimulated rat splenocytes in RPMI 1640 containing 100 ml normal rat serum/l. This preparation was arbitrarily designated to contain 100 units IL-2/ml. The thymidine uptake of CTLL-2 cells was uninfluenced by concentrations of up to 250 ml of normal or zinc-deficient rat sera/l and, at maximal (i.e. 1:1) concentrations of the standard rat IL-2 preparation, consistently produced thymidine uptakes of 25 000–30 000 counts/min.

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Table 2. *Effects of zinc deficiency on rat spleen weights, adherent and non-adherent splenocytes and T-cell subsets binding monoclonal antibodies*

(Mean values and standard deviations for eight rats per group)

	Zn deficient		Pair-fed		Statistical significance of difference: *P*
	Mean	SD	Mean	SD	
Spleen wt (g)	0·25	0·06	0·40	0·03	< 0·01
Spleen wt/kg body-wt (g)	2·1	0·4	2·4	0·3	NS
No. of splenocytes recovered (/g spleen ($\times 10^6$))	220	116	343	143	NS
Percentage adherent cells	50·4	9·0	37·0	11·0	< 0·05
No. of non-adherent splenocytes (/g spleen ($\times 10^6$))	114·7	79·3	219·8	105·3	< 0.05
Percentage non-adherent cells labelled with					
W3/13-positive cells (total T-cells)	63·6	7·6	67·8	8·4	NS
W3/25-positive cells (helper-inducer T-cells)	38·9	8·8	43·8	7·3	NS
Ox8-positive cells (cytotoxic-suppressor T-cells)	32·3	5·0	35·0	5·7	NS
No. of non-adherent cells labelled with (/g spleen ($\times 10^6$))					
W3/13-positive cells	75·2	60·5	144·3	68·8	< 0·05
W3/25-positive cells	46·8	36·8	94·6	43·8	< 0·05
Ox8-positive cells	39·4	36·1	81·4	51·3	< 0·05

NS, not significant.

Statistical analyses

Values from the two groups of rats were compared by the analysis of variance after normalization by log transformation where necessary. Where values are summarized as means (and SD), no statistical conclusions have been drawn from these expressions.

RESULTS

After 4 weeks, growth was significantly impaired in the Zn-deficient group (mean weight 120 (SD 11·4) g) compared with the pair-fed control group (mean weight 169 (SD 12·2) g) ($P < 0·01$). This was a reflection of the successful induction of Zn deficiency in the Zn-restricted animals (mean plasma Zn 11 (SD 3·5) μmol/l) compared with the animals receiving a normal diet (mean plasma Zn 23·7 (SD 1·7) μmol/l).

Effects of Zn deficiency on peripheral blood lymphoid cells

The values summarized in Table 1 indicate that Zn deficiency produced a significant leucopenia. This appeared to affect the lymphocytic component of the total circulating leucocyte count markedly, with little effect on the monocytic and neutrophil compartments. There did not appear to be a selective loss of any single T-lymphocyte subset within the total peripheral blood lymphocyte population since the relative percentages of cells binding the McAB W3/13, W3/25 and Ox8 were similar between the two groups of animals. As might be expected from the general lymphocytopenia produced in the Zn-deficient rats, total circulating numbers of cells binding each of the McAB were significantly diminished.

Effects of Zn deficiency on splenic lymphoid cells

Splenic weights in the Zn-deficient rats were significantly lower than in the normal rats (Table 2) but not when expressed as a proportion of the body-weight. A greater number of cells were obtained from the spleens of the normal rats than from those of the Zn-deficient rats. The percentage of plastic-adherent cells were greater in the Zn-deficient rats than in

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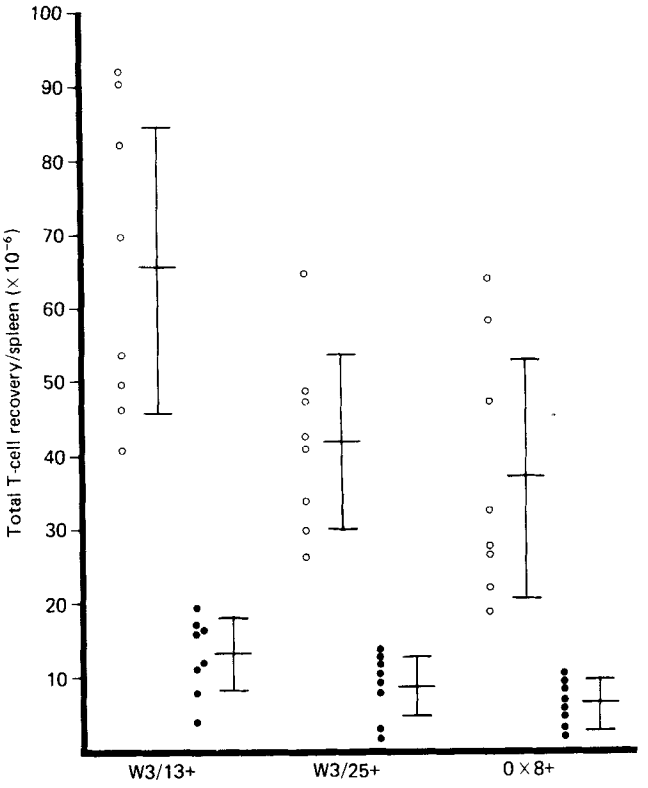

Fig. 1. Total numbers of non-adherent spleen cells binding the monoclonal antibodies W3/13 (total T-cells), W3/25 (helper-inducer T-cells) and Ox8 (cytotoxic-suppressor T-cells) in normal (○) and zinc-deficient (●) rats. Vertical bars denote mean and SD of values for each group.

pair-fed controls (50·9 (SD 9)% *v.*37 (SD 11)%, $P < 0{\cdot}05$), again demonstrating lymphocyte depletion. However, the percentages of non-adherent cells labelling with each of the McAB were not significantly different between the two groups. Although the total number of cells per spleen labelled with each antibody was significantly lower in the Zn-deficient rats (Fig. 1), there was no indication that Zn deficiency exerted a selective influence on the T-cells with any particular phenotype.

The SI of rat splenocytes on stimulation with Con-A in the presence of normal and Zn-deficient serum are shown in Fig. 2. The mean SI in normal serum of 491 (SD 223) for splenocytes of rats receiving a normal diet was almost identical to the mean of 500 (SD 215) for the splenocytes of Zn-deficient rats when cultured under identical conditions. Similarly, when culture was performed in 100 ml Zn-deficient rat serum/l, a mean SI of 351 (SD 153) was obtained for cells from normal rats compared with a value of 393 (SD 157) for the splenocytes of Zn-deficient rats. It is readily seen that a very wide range of thymidine uptake results was obtained in these experiments in both groups of animals as exemplified by a mean radioactivity (counts/min) for normal rat lymphocytes cultured in normal serum of 113 536 (SD 47 369) compared with 128 521 (SD 37 983) for Zn-deficient splenocytes cultured under identical conditions. However, although not statistically significant for the Zn-deficient rats, there was a significant increase in SI on culture of normal rat splenocytes in normal serum compared with culture in Zn-deficient serum ($P < 0{\cdot}05$) and when the results for all

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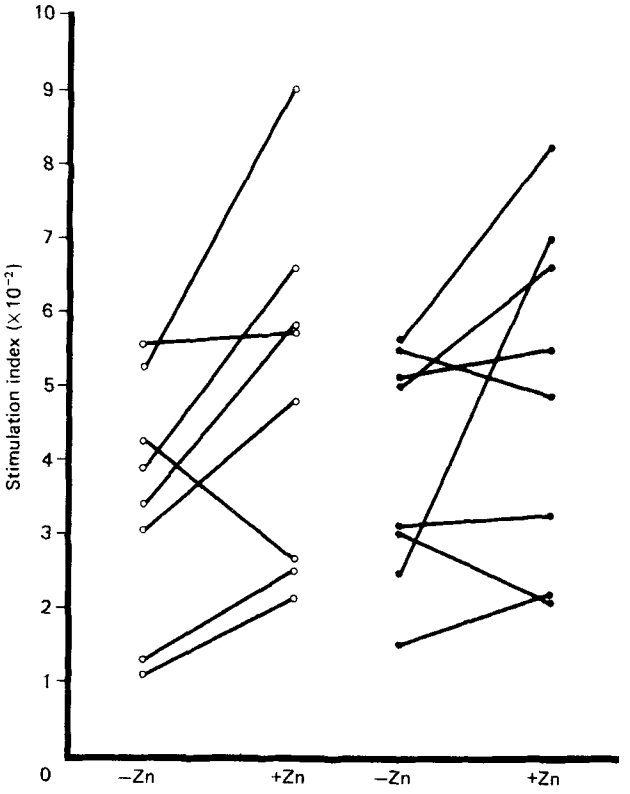

Fig. 2. Stimulation indices ([³H]thymidine uptake in Concanavalin-A-stimulated cultures (counts/min) ÷ [³H]thymidine uptake in unstimulated cultures) of splenic non-adherent cells from normal (○) and Zn-deficient (●) rats on cultures of the same cells in either normal (+Zn) or Zn-deficient (−Zn) serum.

sixteen rats were combined the increase in SI consequent upon culture in normal serum, compared with Zn-deficient serum, was highly significant ($P < 0{\cdot}01$).

The IL-2 content of supernatant fractions from Zn-deficient and normal Con-A activated rat splenocytes in the presence of normal or Zn-deficient serum are shown in Fig. 3. The mean IL-2 content of cells from normal rats, when cultured in normal serum, was 137·3 (SD 67·4) units/ml. The IL-2 content of supernatant fractions from the same cells was in all instances lower when culture was performed in Zn-deficient serum (mean 106·5 (SD 99·4) units/ml, $P < 0{\cdot}01$). In contrast, the IL-2 content of supernatant fractions from Zn-deficient rat splenocytes cultured in Zn-deficient serum (mean 224·8 (SD 39·7) units/ml) was significantly higher than that of normal rat cells whether cultured in Zn-deficient ($P < 0{\cdot}002$) or normal serum ($P < 0{\cdot}01$). However, in all but one instance, culture of splenocytes from Zn-deficient rats in the presence of normal serum resulted in a reduction of the IL-2 content of the supernatant fraction (mean 167·8 (SD 39·7) units/ml) compared with that observed on culture in the presence of Zn-deficient serum ($P < 0{\cdot}01$).

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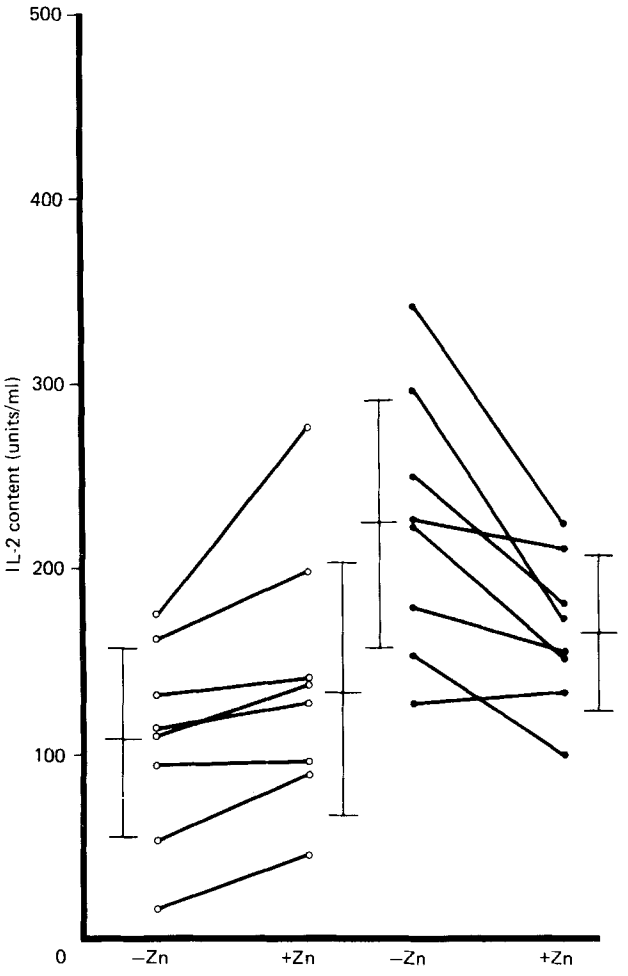

Fig. 3. Interleukin-2 (IL-2) content of supernatant fractions from normal (○) and zinc-deficient (●) Concanavalin-A-activated splenocyte cultures in the presence of normal (+Zn) and Zn-deficient (−Zn) serum. Vertical bars denote mean and SD of values for each group of results.

DISCUSSION

Immune responses are impaired by both deficiency and excess of Zn (Bach, 1981; Chandra, 1984). The immunological consequences of clinical and experimental Zn deficiency have been repeatedly studied with results similar to some reported here (Chandra *et al.* 1980; Allen *et al.* 1981; Malave & Benaim 1984). In particular our Zn-deficient rats exhibited a marked leucopenia with splenic atrophy. Of the cellular components of these two compartments of the lymphoid system, the brunt of this atrophy was borne by the lymphocytes as evidenced by an increase in the percentage of peripheral blood neutrophils concomitant with a decrease in the percentage lymphocytes seen in peripheral blood smears. Similarly there was a greater percentage of adherent (non-lymphoid) cells in the spleen of Zn-deficient rats than in those of the pair-fed normal rats, this being accompanied by gross reductions in the percentage of non-adherent cells in such spleens, thus explaining the fact that total splenocytes recovered per spleen were similar in the two groups.

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This morphologically-defined lymphoid atrophy was confirmed at the phenotypic level by finding marked reductions in the numbers of lymphocytes binding the McAB W3/13 (total T-cells) in both peripheral blood and spleens of Zn-deficient animals. However, there did not appear to be any differential effect of Zn deficiency on the T-cell subsets binding the McAB with specificity either for the helper-inducer (W3/25-positive) or cytotoxic-suppressor (Ox8-positive) T-cell subsets. Thus, these findings do not support the contention of Gross *et al.* (1979*a*) and Fraker *et al.* (1978) that Zn deficiency preferentially impairs helper T-cell development.

Our findings concerning the mitogenic response of non-adherent splenocytes to stimulation with the mitogen Con-A are also at variance with the reports of Gross *et al.* (1979*a*, *b*) insofar as we have been unable to confirm a significant difference between the thymidine uptake of Zn-deficient rate splenocytes and that of splenocytes from normal rats. However, an important methodological difference exists between our studies and those of others in that we have examined the mitogen responses of non-adherent splenocytes whereas previous reports have employed non-separated cell populations. It is now well-recognized that depletion of adherent cells from rodent splenocytes results in significant increases in DNA synthesis in culture (Folch *et al.* 1973; Baird & Kaplan, 1977). However, supplementation of culture medium with Zn has been reported to exert a positive regulatory effect on the murine T-cell response to polyclonal mitogens (Malave & Benaim, 1984) and this also appears to be the case with our studies. No difference in mitogenic response between non-adherent cells from Zn-deficient or normal rats could be detected when the cells from both groups were cultured under identical conditions. There was, however, a significant increase in DNA synthesis by the cells of both groups when culture was conducted in the presence of normal serum as compared with culture in Zn-depleted serum. These findings highlight the important conclusions of Messer *et al.* (1982) on the importance of the trace element content of media employed in such studies.

Further emphasis of this point is provided by the unexpected results obtained on examining the IL-2 content of the culture medium of normal and Zn-deficient rat splenocytes cultured in the presence of normal or Zn-deficient serum. IL-2 is a 15 000 molecular weight lymphokine primarily associated with T-cell proliferation (Robb, 1984). It is released by T-cells during antigenic- or lectin-stimulation and binds to receptors on activated T-cells whose proliferation is then sustained. It therefore plays an essential role in T-cell-mediated immune responses. The IL-2 content of the supernatant fraction from a given stimulated T-cell population can only reflect the net result between the rate of IL-2 production and the rate of IL-2 binding by specific receptors on activated T-cells. It has previously been reported that during Zn deficiency the production and release of lymphokines other than IL-2 are impaired (Bendtzen, 1980; Salvin & Rabin, 1984). In our studies the IL-2 content of supernatant fractions from splenocytes of both normal and Zn-deficient rats was similar when the T-cells were stimulated in the presence of serum with a normal Zn content. However, when normal rat splenocytes were cultured in the absence of Zn, IL-2 content of supernatant fractions was lower than when the activation was performed in serum with a normal Zn content. Conversely, however, the supernatant fractions of Zn-deficient rat splenocytes cultured in Zn-deficient serum appeared to contain higher levels of IL-2 than those of normal rat cells whatever the conditions of culture. These findings suggest that in vitro, Zn is necessary not only as a co-factor for the generation of IL-2 itself but also for the synthesis of cell-surface receptors for IL-2. According to such an interpretation, an intracellular deficiency of Zn, whilst allowing the generation of at least some IL-2, prevents the normal elaboration of IL-2 receptors. Culture in a normal Zn-containing medium reverses the intracellular Zn deficiency and restores IL-2 receptor formation.

It is now widely recognized that even marginal Zn-deficiency produces reduced serum

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levels of the biologically active form of the thymic hormone thymulin which is normally present as a complex with Zn (FTS-Zn Facteur Thymique Serique) (Iwata *et al.* 1979; Chandra *et al.* 1980; Dardenne *et al.* 1984). Thymulin is a necessary co-factor for the maturation of T-cells and its reduced level in Zn-deficient animals may also explain the findings described previously. However, it would also have to be postulated that IL-2 receptor formation was thymulin dependent in order to explain the reduction in IL-2 content consequent on replacing the Zn-deficient serum by normal serum in studies employing Zn-deficient rat splenocytes. To date there have been no specific studies on the influence of thymulin on IL-2 receptors although it is generally conceded that thymic hormones act on the cell surface membrane to alter receptor sites and affinity binding of pre-committed lymphocytes (Bach *et al.* 1978). Further investigation of this suggestion might be facilitated by the recent development of McAB to the IL-2 receptor (Osawa & Diamantstein, 1984).

In summary, these studies confirm the T-cell dominance of the lymphoid atrophy which accompanies Zn deficiency in the rat. Functional studies indicate that T-cells from both Zn-deficient and normal rats behave normally in response to mitogenic stimulation when cultured in the presence of normal rat serum. Culture in Zn-deficient serum, however, produces impaired responses as estimated by crude thymidine uptake but the synthesis of the important lymphokine IL-2 and its receptors by activated T-cells may be determined by the pre-existing intracellular Zn concentration.

We would like to thank Mrs C. W. Ramsden for her invaluable technical assistance and Mrs I. E. Poole for all her secretarial help. P. S. D. is supported by the Manny Cussins Research Fellowship jointly administered by the West Riding Medical Research Trust and the British Digestive Diseases Foundation.

REFERENCES

Allen, J. I., Kay, N. E. & McClain, C. J. (1981). *Annals of Internal Medicine* **40**, 154–157.
Bach, J. F. (1981). *Immunology Today* **2**, 225–227.
Bach, J. F., Bach, M. A., Blanot, D., Bricas, E., Charreire, J., Dardenne, M., Fournier, C. & Pleau, J. M. (1978). *Bulletin of the Pasteur Institute* **76**, 325–335.
Baird, L. G. & Kaplan, A. M. (1977). *Cellular Immunology* **28**, 22–29.
Bendtzen, K. (1980). *Scandinavian Journal of Immunology* **12**, 203–209.
Cantrell, D. A., Robins, R. A., Brooks, C. G. & Baldwin, R. W. (1982). *Immunology* **45**, 97–103.
Chandra, R. K. (1984). *Journal of the American Medical Association* **252**, 1443–1446.
Chandra, R. K., Heresi, G. & Au, B. (1980). *Clinical and Experimental Immunology* **42**, 332–335.
Dardenne, M., Savino, W., Wade, S., Kaiserlian, D., Lemonnier, D. & Bach, J. F. (1984). *European Journal of Immunology* **14**, 454–458.
Fernandes, G., Nair, M., Onoe, K., Tanaka, T., Floyd, R. & Good, R. A. (1979). *Proceedings of the National Academy of Sciences of the USA* **76**, 457–461.
Folch, H., Yoshinaga, M. & Wakeman, B. H. (1973). *Journal of Immunology* **110**, 835–838.
Fraker, P. T., Deposquale-Jardieu, P., Zwickle, L. M. & Leucke, R. W. (1978). *Proceedings of the National Academy of Sciences of the USA* **75**, 5660–5664.
Fraker, P. T. & Leucke, R. W. (1981). *Advances in Experimental Medicine and Biology* **135**, 107–119.
Frost, P., Rabberi, P., Smith, T. & Prasad, A. (1981). *Proceedings of the Society for Experimental Biology and Medicine* **167**, 333–337.
Gillis, S. (1983). *Journal of Clinical Immunology* **3**, 1–13.
Gillis, S., Ferm, M. M., Ou, W., & Smith, K. A. (1978). *Journal of Immunology* **120**, 2027–2032.
Gross, R. L., Osdin, N., Fong, L. & Newberne, P. M. (1979*a*). *American Journal of Clinical Nutrition* **32**, 1260–1265.
Gross, R.L., Osdin, N., Fong, L. & Newberne, P. M. (1979*b*). *American Journal of Clinical Nutrition* **32**, 1267–1271.
Guillou, P. J., Kerr, M. B., Ramsden, C. & Giles, G. R. (1984). In *New Perspectives in Theophylline Therapy* (International Symposium series no. 78), pp. 157–164 [M. Turner-Warwick and J. Levy, editors]. London: Royal Society of Medicine.
Iwata, T., Incefy, G. S. & Tanaka, T. (1979). *Cellular Immunology* **47**, 100–109.
Kohler, G. & Milstein, C. (1975). *Nature* **256**, 495–497.

https://doi.org/10.1079/BJN19860010 Published online by Cambridge University Press

Malave, I. & Benaim, I. R. (1984). *Cellular Immunology* **89**, 322–330.
Messer, H. H., Murray, E. T. & Goebel, N. K. (1982). *Journal of Nutrition* **112**, 652–657.
National Academy of Sciences (1972). *Nutrient Requirements of Laboratory Animals*, publication no. 10. Washington DC: National Academy of Sciences.
Osawa, H. & Diamantstein, T. (1984). *European Journal of Immunology* **14**, 374–377.
Robb, R. J. (1984). *Immunology Today* **5**, 203–209.
Salvin, S. B. & Rabin, B. S. (1984). *Cellular Immunology* **87**, 546–552.
Williams, A. F., Galfre, G. & Milstein, C. (1977). *Cell* **12**, 633–673.

Printed in Great Britain

https://doi.org/10.1079/BJN19860010 Published online by Cambridge University Press