S-18-2 Zinc Status in Proliferative Response of T Lymphocytes

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I. INTRODUCTION

In man and animals in zinc (Zn) deficiency or with hereditary malabsorption of Zn, immunological disturbance as well as growth retardation has been described, including involution of thymus and lymphoid organs and increased susceptibility to infection [1,2]. Such immunological disturbance are restored by oral or parenteral administration of Zn compounds. Since the first observation on dysfunction of cell-mediated immunity in Zn-deprived mice [3], experimental studies with dietary Zn deficiency as a single nutritional variable have shown a significant depression of T cell proliferation and function as shown in Table 1 [4]. Zn is thus considered as a critical biofactor to regulate T cell-dependent immune response through Zn-dependent immune processes. An in vitro experiment moreover shows that proliferative response of T cells are more susceptible to Zn deprivation with chelating agent compared with that of B cells [5]. It is much of interest why T cells are highly susceptible to deprivation of Zn.

Although lymphocyte proliferation requires Zn as an essential nutrient [6], the precise mechanism for Zn-dependent immune processes, in particular the selective requirement of Zn by T cells, is not clear. Recent findings indicate that Zn plays a critical role in activation of a thymic hormone, thymulin, which promotes proliferation and differentiation of immature thymocytes [7]. On the other hand, Zn is well known as a cofactor of many enzymes and transcription factors necessary for DNA replication and RNA transcription, e.g. DNA and RNA polymerases, thymidine kinase and transcription factor A [8]. Moreover, Zn has been suggested to regulate signal transduction in T cells through activation and translocation of protein kinase C [9,10]. However, these are not enough to explain the selective dependency of matured T cells to Zn.

We find that proliferative response of cultured mouse spleen cells to T cell mitogens, concanavalin A (Con A) and phytohemagglutinin (PHA), are more

TABLE 1.

Immunological alterations caused by nutritional zinc restriction as a single nutritional variable.

1. Deficiency of T cell number
 2. Reduced proliferative responses of T cells to phyto mitogens, allogeneic cells and common antigens
 3. Decreased T cell-dependent antibody production, e.g. in primary antibody response to sheep red blood cells
 4. Decreased tempo of skin allograft rejection
 5. Decreased natural killer cell activities
 6. Decreased functional activity levels of thymulin
 7. Decreased accessory function of macrophages for proliferative response of T cells to a phyto mitogen (PHA)
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susceptible to cadmium (Cd) than that to B cell mitogen, lipopolysaccharide (LPS) [11]. The differential susceptibility to Cd of mitogen responsiveness between T and B cells is similar to that to the *in vitro* Zn deficiency [5]. In fact the inhibition of T cell proliferation by Cd are protected by simultaneous addition of Zn [12]. This suggests that Zn-dependent processes in proliferative response of T cells can be the sensitive target(s) of Cd toxicity. Then, in order to define Zn status in T cell proliferation, we further studied toxicological situation of Zn in proliferative responses of T cells to Con A [13], and also have done a biochemical approach to detect Zn-dependent molecules induced during T cell proliferation.

II. ZINC STATUS IN THE SUSCEPTIBILITY OF PROLIFERATIVE RESPONSE OF T LYMPHOCYTES TO CADMIUM

1. Specific Implication of Zinc in the Susceptibility of T Cell Proliferation

First, we tried to characterize the differential susceptibility to Cd of mitogen responsiveness between T and B cells in cultured spleen cells of BALB/c mice. Results are obtained as follows: (1) the differential susceptibility to Cd is observed on both DNA and RNA synthesis induced by mitogens, Con A (2 µg/ml) and LPS (20 µg/ml). (2) High susceptibility of T cell proliferation is characteristic of Cd, because in the comparative study with various heavy metal compounds there were no similarities in their effects on the mitogen-induced DNA synthesis between Cd and other heavy metals such as Pb, Hg(II), Ni, Mo, Ag, Cr(VI) and As(V). (3) The early events during the response to Con A, such as activation of macrophages and helper T cells, are unlikely susceptible to Cd, because 10 µM Cd did not affect interleukin-2 production by activated helper T cells precedent to T cell proliferation. (4) The inhibition by Cd at around 10 µM of Con A-induced proliferative response of T cells was mostly protected by simultaneous addition of 30 µM Zn. The protection from the inhibitory effect of Cd was obtained only with Zn among 4 essential divalent metals: Zn, Cu, Fe and Ni. These findings indicate that Zn-dependent processes are specifically implicated in the susceptibility of T cell proliferation to Cd.

2. The Zinc-dependent Processes Required for T Cell Proliferation Should be Targets Susceptible to Cadmium

There are two possible explanations for the protective effect of Zn on the inhibitory action of Cd to T cells: first, Zn may decrease effective concentration of Cd in T cells, through reduction of Cd uptake or induction of metallothionein which binds Cd. Second, Zn may compete with Cd on Zn-dependent processes required for T cell proliferation. To estimate these possibilities, we examined effect of Zn added at the protecting dose on cellular uptake and distribution of ¹⁰⁹Cd (Cd in whole cell, cytoplasm and crude nuclei, and metallothionein-bound Cd) in spleen cell cultures. In spleen cells at 24 h after Con A-stimulation the addition of Zn at 30 µM could neither reduce the intracellular Cd content nor promote induction of Cd-thionein. This indicates that the protective effect of Zn is not due to a decrease in the effective concentration of Cd in proliferating T cells, but rather due to competition between Zn and Cd in the Zn-dependent processes of T cell proliferation. To further elucidate the Zn-dependent processes in the proliferative response of T cells, we determined the effective time point of the inhibitory action of Cd or the protecting action of Zn against Cd. Cd was added to spleen cell cultures at various time-points after Con A addition, and ³H-thymidine uptake during the final 4 hr of 48 hr culture was determined. Cd effectively inhibited the thymidine uptake when added at 16 h after Con A stimulation, and thereafter the effect gradually decreased. The protecting effect of Zn against Cd action was also effective within first 16 h after the mitogen stimulation. Con A-induced thymidine uptake by spleen cells began 24 h after the mitogen stimulation, and then most of proliferating T cells stay

before the S phase of cell cycle at 16 h of the mitogen stimulation. These findings indicate that the Zn-dependent sites expressed before the S phase of cell cycle are susceptible to Cd and critical for T cell proliferation.

Thus the Zn-dependent processes required for T cell proliferation, which are expressed before the S phase of cell cycle, can be target(s) of the differential toxicity to T cells. Zn enzymes such as DNA polymerase and thymidine kinase are well discussed in relation to Zn requirement in cell proliferation. The Cd-induced inhibition of uridine uptake in T cells however begins between 10 and 16 h after Con A stimulation. Then it is most likely that the Zn-dependent processes responsible for susceptibility of T cell proliferation occur not on Zn enzymes involved in DNA synthesis but on some molecules activated or induced in the G1 phase followed by induction of DNA synthesis.

III. DETECTION OF ZINC-BINDING PROTEINS IN CONCAVALIN A-STIMULATED T LYMPHOCYTES

Many different proteins appear or disappear during T cell proliferation [14]. Some of them may be induced specifically during mitogen stimulation of T cells, and require their association with Zn for processing proliferative response. We have therefore detected Zn-binding proteins in mouse spleen cells stimulated with Con A. ⁶⁵Zn-blotting analysis of the cytosol fraction from Con A-stimulated spleen cells for 24 h showed induction of three Zn-binding proteins with approximate molecular weight of 49, 90 and 100KD typically recognized among many proteins on SDS-polyacrylamide gel. When cultured spleen cells were labeled with ⁶⁵Zn in medium, Zn-binding proteins of 49 and 100KD also were detected in Con A stimulated cells. Electrophoretic features of these two proteins with high affinity to Zn are different from those of some authentic Zn enzymes, and seemingly from those of protein kinase C of about 80KD. It is unknown whether they are the equivalents of lymphoid specific tyrosine kinase p56^{lck} enhanced by Zn [15] and 11.5KD Zn-binding protein, parathymosine [16], respectively. It is conceivable that these Zn-binding proteins play a role as a target of Zn action in T cell proliferation.

In conclusion, our findings elucidate that Zn plays a critical role in regulation of T cell proliferation at the processes expressed before the S phase, and suggest that the Zn-dependent processes can be associated with specific Zn-binding proteins induced during proliferative response of T cells. Further study is required to explain biological significance of these Zn-binding proteins in regulation of T cell proliferation.

SUMMARY

The in vitro proliferation of T lymphocytes are highly susceptible to Zn deprivation and Cd addition. In order to define Zn status in T cell proliferation, toxicological and biochemical situation of Zn in proliferative response of T cells was investigated by use of mouse spleen cell cultures stimulated by T cell-mitogen, Con A. The inhibitory effect of Cd on T cell proliferation was protected specifically by Zn. The protection by Zn was effective when Zn was added within 16 h after Con A stimulation. Zn addition affected neither Cd content in cells nor induction of Cd-thionein. These findings indicate that Zn-dependent processes expressed before the S phase of cell cycle are critical for T cell proliferation and are targets susceptible to Cd. Moreover, electrophoretical analysis showed two unknown Zn-binding proteins (49 and 100KD) induced in spleen cells incubated with Con A and radioactive Zn. These Zn-binding proteins may be associated with the Zn-dependent processes critical for T cell proliferation.

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