Zinc Depletion Modifies CD5 Expression by 70Z/3 Murine Pre-B Leukemia Cell Line (43319)

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Abstract. **CD5 expression on B cells is regulated by certain humoral factors. In a pre-B leukemia cell line 70Z/3, we found that interleukin 4 down-regulates it. Herein, we report that zinc influences spontaneous CD5 expression by this cell line as well as actions of these factors on CD5 expression considerably.**

In zinc-depleted culture media, spontaneous CD5 expression by 70Z/3 cells was enhanced. In contrast, the down-regulatory action of interleukin 4 was significantly reduced under culture conditions of zinc depletion. The supplementation of zinc to physiologic concentrations (1 to 2 μM) abolished such effects of zinc-depleted medium. The reduction of the suppressive action of interleukin 4 was observed at the level of gene expression. However, CD5 mRNA expression enhanced by lipopolysaccharide or NZB-SF was not further enhanced under conditions of zinc deficiency. These observations may suggest that CD5 expression by malignant or even normal B cells may be influenced by cellular/serum zinc levels. [P.S.E.B.M. 1991, Vol 198]

The expression of cell surface molecules is becoming more and more clearly implicated in the differentiation and function of cells of various lineage cells. However, the roles played by many cell surface markers remain obscure. Among such cell surface molecules whose functions remain unknown is the rather unique CD5 antigen. This antigen was initially described as a pan T cell marker, but during the past decade the existence of $CD5^+$ B cells as a subpopulation of B lymphocytes has been well established (1–4). A minor B cell subset bearing CD5 antigen has been shown to be expanded in certain autoimmune-prone strains of mice, particularly mice of NZB background as well as in patients who exhibit autoimmune disorders (1, 2, 5–7). It is suggested that $CD5^+$ B cells may represent cells at an immature stage of B cell differentiation which produce so-called natural antibodies (1, 8). It is also suggested that these cells are functionally important to provide idiotype-specific activation signals to antigen-specific $CD5^-$ B cells (9).

Recently, several humoral factors have been shown to regulate CD5 expression on B lineage cells. Interleukin 4 (IL-4) down-regulates CD5 expression on normal and malignant $CD5^+$ B cells in humans, whereas 12-*O*-tetradecanoylphorbol 13-acetate seems to up-regulate CD5 expression (10–12). We have shown that CD5 expression by cells of the 70Z/3 murine pre-B leukemia cell line can be up-regulated by lipopolysaccharide (LPS) and/or NZB serum factor (NZB-SF), a B cell maturation factor originally isolated from mice of the autoimmune-prone NZB strain (13, 14). Furthermore, CD5 expression by this cell line is also down-regulated by IL-4 (15).

On the other hand, zinc is known to be a crucial nutritional component required for normal development and maintenance of the immune functions (16, 17). Recent progress in molecular biology has also revealed that this mineral is crucial for the function of many DNA-binding proteins (so-called zinc fingers) which regulate transcription of a wide range of proteins (18, 19). In fact, Ig κ light chain transcription is regulated by nuclear factor (NF)-κB, one of the zinc finger proteins operational in this cell line (20–23).

In this study we have attempted further to elucidate the mechanism involved in CD5 expression on cells of the 70Z/3 pre-B leukemia cell line and found that zinc appears to exert important roles in the actions of factors involved in expression of CD5 by these cells.

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Materials and Methods

Cell Cultures and Cell Surface Marker Analysis. 70Z/3 murine pre-B leukemic cell line (ATCC, Rockville, MD) was subcloned in our laboratory and maintained in RPMI 1640 supplemented with 10% fetal calf serum (FCS) (Hyclone, Logan, UT), 5×10^{-5} M 2-mercaptoethanol (2-ME), and other additives. When testing the expression of cell surface markers, 70Z/3 cells were incubated for 24 hr in the presence of various humoral factors (2.5×10^5 cells/ml). Then cells were harvested, stained, and subjected to cell surface marker analysis by a flow cytometer (EPICS Elite; Coulter, Miami, FL). CD5 antigen was stained with anti-Ly-1 monoclonal antibody fluorescein isothiocyanate (FITC) conjugate (Becton-Dickinson, Mountain View, CA). Surface immunoglobulin (sIg) was stained with FITC-conjugated goat anti-mouse IgM antibody ($(Fab)_2$ fragment; Cappel, Westchester, PA). Rat monoclonal B220 antibody was purified from hybridoma (RA3.3A1/6; ATCC) supernatant by precipitating IgM antibody in 0.01 M NaCl (24). AA4.1 antibody was purchased from DuPont, Boston, MA (25). As a second antibody, goat anti-rat Ig-FITC conjugate was used for B220 and AA4.1 antibodies. Ia antigen was detected by staining cells with monoclonal anti-Ia antibody-biotin conjugate (Meiji Health Science, Tokyo, Japan) and streptoavidin-phycoerythrin conjugate (Becton-Dickinson). Preimmunized goat serum-FITC conjugate (Tago, Burlingame, CA) was used as control antibody to detect nonspecific binding. Gating was always adjusted so that nonspecific staining detected by control antibody would be less than 2%. With this gate setting, autofluorescence demonstrated by unstained 70Z/3 cells was always less than 1%. Before staining, 70Z/3 cells were treated with 0.17 M $(NH_4)_2Cl_2$ (pH 7.3 for 3 min to remove dying cells and washed twice with phosphate) buffered saline.

Reagents. Lipopolysaccharide (LPS) (Difco, Baxter Scientific, Ocala, FL) and IL-4 (Genzyme, Boston, MA) was purchased from commercial sources as indicated. NZB-SF was prepared in our laboratory as described earlier (26). Briefly, young NZB mice (4 to 5 weeks old) were injected with 1 mg of *Corynebacterium parvum* intraperitoneally and sacrificed 14 days later. Spleen cell suspensions were cultured for 24 hr in RPMI 1640, 2-ME, penicillin and streptomycin, HEPES, sodium pyruvate, and 1% Nutridoma sp. (Boehringer-Mannheim, Indianapolis, IN). Then NZB-SF was purified by applying supernatant harvested to the membrane affinity capsule conjugated with monoclonal antibody against NZB-SF (Mac 50; MEMTEK, Billerica, MA) (14). After vigorous washing with various buffers, NZB-SF was eluted by 3 M NaSCN and dialyzed immediately. Affinity purified NZB-SF was stored in the presence of 1 mM EDTA, trypsin inhibitor, and 0.2 mM phenylmethyl sulfonyl fluoride at 4°C, or frozen at −20°C in the presence of 0.01% bovine serum albumin. NZB-SF prepared in this way showed a major band of 60 kDa in sodium dodecyl sulfate-polyacrylamide electrophoresis analysis. One unit of NZB-SF was defined as the amount of NZB-SF which gives 50% maximum activity in the assay of colony-forming B cells as described earlier (13). Typically 1 unit of NZB-SF corresponds to 2 to 3 ng of affinity purified NZB-SF.

Preparation of Zinc-Depleted FCS and $ZnCl_2$. FCS was depleted of zinc by dialyzing it 3 days against 10 mM Na_2EDTA (1:150, three changes), 3 days against 100 mM NaBr (1:150, three changes), and 3 days against 150 mM NaCl (1:150, three changes) (26). The cutting molecular weight of the dialyzing membrane employed was 3500. Stock solution of 100 mM $ZnCl_2$ was prepared by mixing 1 volume of 1 M $ZnCl_2$ in 0.1 N HCl and 1 volume of 1 M Na_2HNTA, adjusted to pH 7.0 by addition of 1 M Tris base and diluted to a 100 mM solution to avoid the precipitation (26). Further dilutions were made on the day of the experiment.

Slot Blot and Northern Blot Analysis of CD5 mRNA Expression by 70Z/3 Cells. 70Z/3 cells were incubated in the presence of various humoral factors for 24 hr. After being washed once with phosphate-buffered saline, cells were resuspended in a solution containing 4 M guanidium thiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% sarcosyl, and 0.72% 2-ME. Then RNA was extracted with phenol/$CHCl_3$ and precipitated in isopropanol. Samples then underwent the same steps again and the RNA pellet was washed once with 75% ethanol, air dried, dissolved in 10 mM Tris-HCl, 1 mM EDTA, pH 7.3 (TE buffer), and stored in a freezer at −80°C until used. After being denatured with 1 M glyoxal, RNA samples were slot blotted (Bio-Dot SF microfiltration units; Bio-Rad, Richmond, CA) on nylon membrane (Z probe; Bio-Rad) or size fractionated in 1.2% agarose gel and transferred to nylon membrane for Northern analysis (Vacugene; BRL, Gaithersburg, MD).

The cDNA probe for mouse CD5 was radiolabeled with [^{32}P]dCTP by the oligolabeling method (Oligolabeling kit; Pharmacia-LKB, Uppsala, Sweden). The plasmid vectors inserted with a cDNA probe that codes for mouse CD5 were kindly provided by Drs. H. J. S. Huang and L. A. Herzenberg of Stanford University (27). The cDNA fragment (400 base pairs) coding for CD5 was used in this study. A synthetic oligonucleotide (27-mer) for mouse β actin (Chlonotech, Palo Alto, CA) or the cDNA fragment (400 base pairs) of human β actin (ATCC) were used as control probes. The x-ray films were exposed at −70°C and screened using a scanning densitometer (Pharmacia-LKB). The average peak absorbance of 5 reading points by CD5 and actin probes was obtained and the results were expressed as

the ratio of average peak absorbance by CD5 probe/actin probe in slot blot analysis.

Results

Changes of Cell Surface Marker Expression in the State of Zinc Deficiency *In Vitro*. 70Z/3 cells were cultured for 24 hr in the media supplemented with either untreated FCS or zinc-deficient FCS. The cells were harvested, treated with lysis buffer, washed twice with phosphate-buffered saline, and subjected for the analysis of cell surface marker expression including CD5, sIg, AA4.1, B220, and Ia antigens. More than 95% of 70Z/3 cells expressed AA4.1, B220, and Ia antigens in both regular and the zinc-deficient culture conditions in three separate experiments (Table I). sIg was expressed by about 40% of cells of this subclone of the 70Z/3 cell line which was also not affected in the zinc-deficient culture conditions (Table I). In contrast, spontaneous CD5 expression seemed to be enhanced in the culture conditions of zinc deficiency (Table I). Then the effects of humoral factors which regulate CD5 expression by 70Z/3 cells were also tested in zinc-deficient culture conditions. As shown in Table I, up-regulatory actions of LPS and NZB-SF appeared to be increased, perhaps reflecting the increase of spontaneous CD5 expression. The down-regulatory action of IL-4 seemed to be less effective in the zinc-deficient culture conditions ($P < 0.05$).

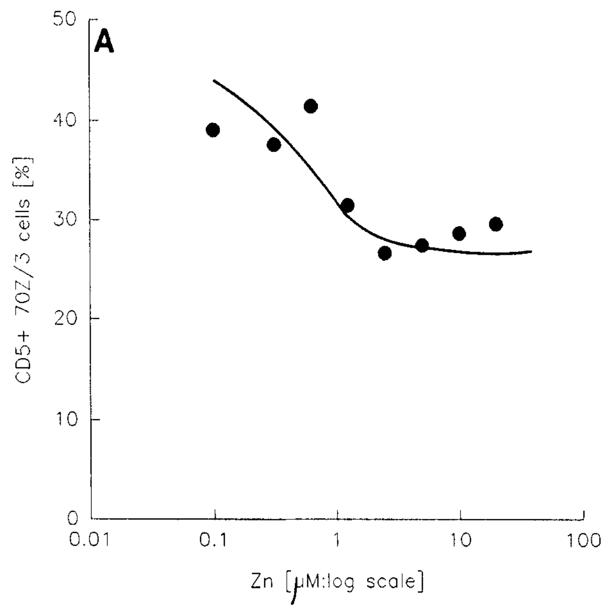
Effects on CD5 Expression by 70Z/3 Cells of Supplementation of $ZnCl_2$ to the Zinc-deficient media. When FCS was dialyzed extensively to remove zinc, it is possible that other components of FCS were also lost during the dialysis. Thus, we could not exclude the possibility that the changes in CD5 expression by 70Z/3 cells cultured in zinc-deficient media may be also related to the loss of components other than zinc. Thus, the effects of zinc-supplementation to zinc-deficient culture medium were also examined. Namely, various doses of $ZnCl_2$ were supplemented to zinc-deficient media and the changes of CD5 expression by 70Z/3 cells were examined in the presence of LPS (1 μg/ml) (Fig. 1A), NZB-SF (10 units/ml) (Fig. 1B), IL-4 (10 units/ml) plus LPS (1 μg/ml) (Fig. 1C), medium only (Fig. 1D), and IL-4 (10 units/ml) (Fig. 1E). As control, the effects of $ZnCl_2$ supplementation to regular medium were also studied. In the presence of more than 0.5 to 1 μM $ZnCl_2$, the enhanced expression of CD5 by 70Z/3 cells observed in zinc-deficient culture conditions was lost regardless of the kind of stimulation employed. That is, in the presence of LPS, NZB-SF, or medium only, the enhanced CD5 expression by 70Z/3 cells cultured in zinc-deficient medium returned to the levels equivalent to those expressed by cells cultured in the regular medium when the latter was supplemented with >0.5 to 1.0 μM $ZnCl_2$. It should be mentioned that NZB-SF which seems to be less effective on CD5 expression by 70Z/3 cells with the concentration employed here (10 units/ml) under the regular culture conditions, manifested a more significant enhancing effect on CD5 expression under conditions of zinc deficiency. The supplementation of $ZnCl_2$ (0.1 to 20 μM) to the regular medium did not change CD5 expres-

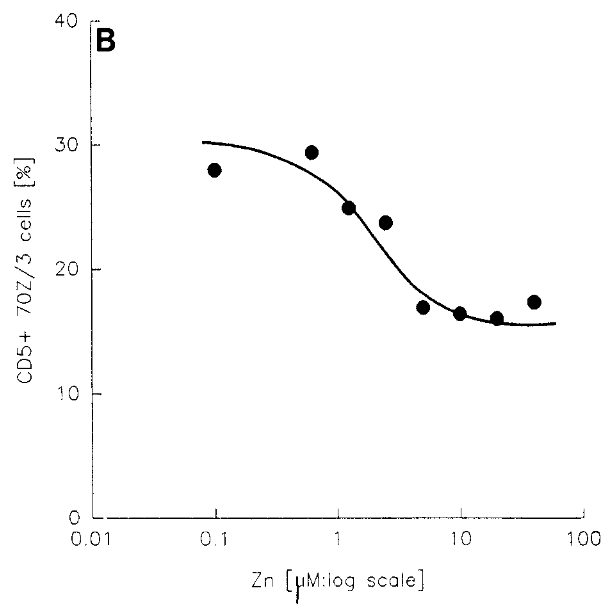
Table I. Changes of Expression of Cell Surface Markers by 70Z/3 Cell Line in the Zinc-Deficient Culture Conditions

Cell surface antigen tested	Cell surface marker expression (%)	
	In regular medium[a]	In zinc-deficient medium[a]
Summary of 3 experiments		
AA4.1	95.0 ± 1.5	96.0 ± 2.1
B220	98.0 ± 2.0	97.5 ± 2.1
Ia	96.0 ± 1.5	95.5 ± 1.5
sIg	45.0 ± 4.5	42.5 ± 2.5
CD5 (medium only)	13.8 ± 1.2	19.0 ± 0.3[b]
CD5 (LPS, 1 μg/ml)	30.8 ± 4.3	38.1 ± 3.4
CD5 (NZB-SF, 10 units/ml)	24.1 ± 5.5	36.3 ± 3.9
CD5 (IL-4, 10 units/ml)	5.2 ± 1.4	10.8 ± 1.2[b]

[a] 70Z/3 cells stained by control antibody as described in Materials and Methods were 1.3 ± 0.4% in the regular medium and 1.1 ± 0.1% in the zinc-deficient culture medium. Autofluorescence presented by unstained 70Z/3 cells were 0.5 ± 0.1% in the regular medium and 0.8 ± 0.1% in the zinc-deficient medium.
[b] CD5 expression is significantly higher when cells were cultured in zinc-deficient medium compared with that when cells were cultured in regular medium ($P < 0.05$).

Figure 1. Changes in CD5 expression by 70Z/3 cells by supplementation of various doses of $ZnCl_2$ when cells were cultured in zinc-deficient medium. 70Z/3 cells were incubated in the presence of LPS, 1 μg/ml (A), NZB-SF, 10 units/ml (B), IL-4, 10 units/ml, plus LPS, 1 μg/ml (C), medium only (D), or IL-4, 10 units/ml (E). CD5 expression by 70Z/3 cells in the absence of zinc in the conditions described above are 41.0%, 31.5%, 27.5%, 22.0%, and 10.1%, respectively. Autofluorescence was less than 1% in these experiments and nonspecific binding detected was 1 to 2% (see Materials and Methods).


A
50
40
30
20
10
0
CD5+ 70Z/3 cells [%]
0.01
0.1
1
10
100
Zn [µM:log scale]


B
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CD5+ 70Z/3 cells [%]
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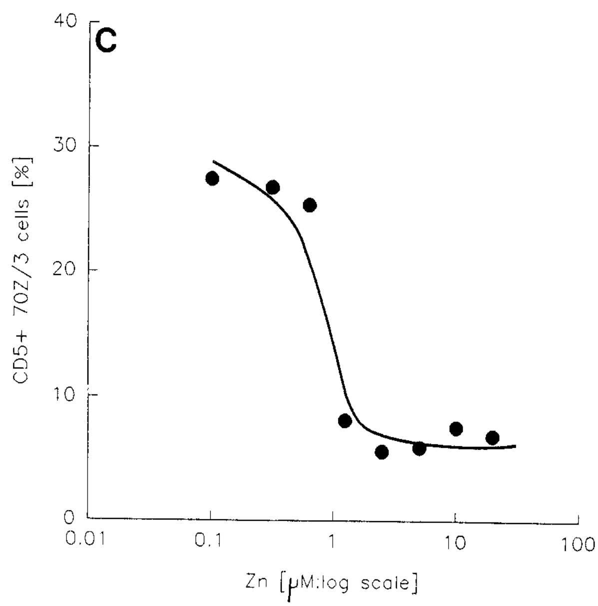
Zn [µM:log scale]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CD5+ 70Z/3 cells [%]
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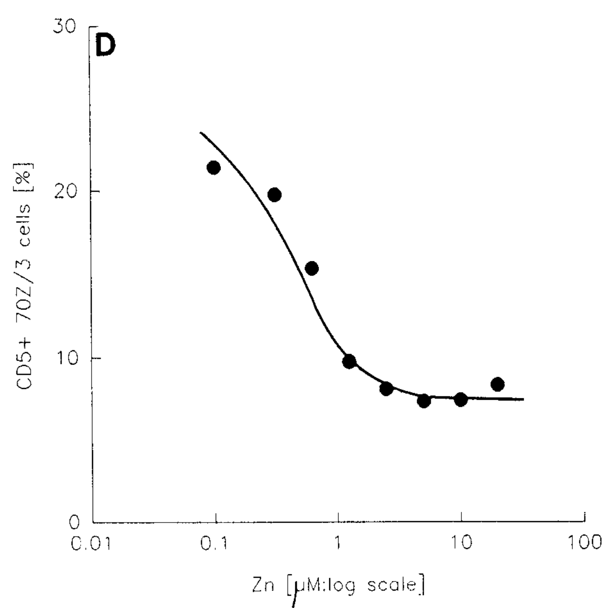
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CD5+ 70Z/3 cells [%]
0.01
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Zn [µM:log scale]

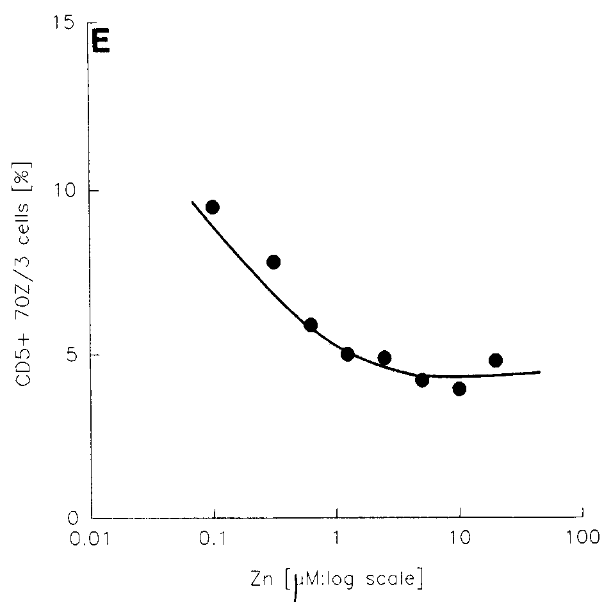
E
15
10
5
0
CD5+ 70Z/3 cells [%]
0.01
0.1
1
10
100
Zn [µM:log scale]

Table II. CD5 Expression by Metabolically Inactive 70Z/3 Cells are Unchanged Irrespective to the Presence of Zinc in the Culture Medium

70Z/3 cells were cultured with	CD5 expression by 70Z/3 cells (%)	
	In regular medium[a]	In zinc-deficient medium[a]
Summary of 3 experiments		
—	12.1 ± 2.1	21.0 ± 1.0
Cycloheximide (3 μg/ml)	15.0 ± 1.5	14.1 ± 2.0
Actinomycin D (5 μg/ml)	14.5 ± 1.0	13.8 ± 1.0

[a] 70Z/3 cells stained by control antibody were 1.5 ± 0.4% in the regular medium and 1.3 ± 0.2% in zinc-deficient medium. Autofluorescense demonstrated by unstained 70Z/3 cells were 0.5 ± 0.1% and 0.4 ± 0.2%, respectively, in these two culture conditions.

sion on 70Z/3 cells (data not shown). Furthermore, the suppressive actions of IL-4 were clearly restored by supplementation of more than 1 μM $ZnCl_2$, a level which is close to physiologic serum levels of zinc (Fig. 1, C and E). These results indicate that the changes in the action of humoral factors on CD5 expression in zinc-deficient medium is mainly due to the depletion of zinc. Then we further tested whether CD5 expression by metabolically inactive 70Z/3 cells can be influenced by the absence of zinc in the culture media. 70Z/3 cells were cultured in the zinc-deficient culture media or in the regular media in the presence of a transcription inhibitor (actinomycin D, 5 μg/ml) and a protein synthesis inhibitor (cycloheximide, 3 μg/ml) and subjected for CD5 expression by flow cytometry. We found that CD5 expression by metabolically inactive 70Z/3 cells was not altered by the absence of zinc in the culture medium (Table 2), indicating that changes of CD5 expression by extracellular zinc may not be related to the changes of bindings of CD5 molecules to anti-CD5 monoclonal antibody.

Effect of the Depletion of Zinc on the Levels of CD5 mRNA. In this study we also examined the changes of CD5 mRNA expression under conditions of zinc depletion. 70Z/3 cells were incubated overnight (16 hr) in zinc-deficient medium with or without zinc supplementation (10 μM) and total cytoplasmic RNA was extracted. In slot blot analysis, 1 to 2 μg of RNA was blotted and hybridized with a radiolabeled CD5 probe. The density of the blot detected by autoradiography was scanned by densitometer as described in Materials and Methods and expressed as the ratio of peak absorbance of CD5/actin probe. As shown in Figures 2 and 3, under culture conditions of zinc depletion, CD5 mRNA levels were not significantly decreased in the presence of IL-4 in contradistinction to regulate culture conditions (15). However, the suppressive action of IL-4 on CD5 mRNA levels was readily restored when the culture medium was supplemented with $ZnCl_2$ (10 μM). By contrast, zinc restriction or zinc supplementation did not change the enhancing influences of LPS or NZB-SF on CD5 mRNA levels (Fig. 2). In northern blot analysis samples of total RNA (5 μg of each) from 70Z/3 cells were employed. 70Z/3 cells were cultured in the presence of IL-4 (10 units/ml) in the regular medium, in the zinc-deficient medium, and in the zinc-deficient medium supplemented with $ZnCl_2$ (10 μM). We obtained similar results to those obtained with the slot blot analysis (Fig. 3). That is, the suppressive effect of CD5 mRNA expression by IL-4 was not clearly observed in zinc-deficient culture conditions which were restored by the supplementation of $ZnCl_2$ (10 μM).

Discussion

Beginning with the initial work of Prasad and his colleagues (16, 17), it has been well established that zinc deficiency can result in profound immune dysfunctions, especially T cell dysfunctions. Thymulin, one of the thymic hormones important for maturation of T lineage cells, requires zinc for its biologic function and may partly account for T cell dysfunctions observed in patients with a zinc deficiency (16, 28, 29). The recent progress in molecular biology has also revealed the presence of the family of DNA-binding proteins requiring zinc for their structure and function—so-called zinc fingers (18, 19), which are regulators of transcription of various proteins and may act as a second messenger for signals transmitted to the cells by hormones or other stimulators.

Over the past decade the presence of $CD5^+$ B cells, as well as their unique characteristics, were also well established (1–4, 9). $CD5^+$ B cells appear to represent a rather unique B cell lineage, producing polyclonal low-affinity IgM antibodies spontaneously including autoantibodies and repeatedly using the highly conserved V_H and/or V_L genes (1–4, 9). They are also characterized as being poorly responsive to specific antigenic stimulation (9). Some authors reported that $CD5^+$ B cells may represent a subset of immature B lineage cells which in turn restrict the expansion of self-reactive B cell clones (30). Others reported that these autoreactive cells may be advantageous for a normal immune system by providing idiotype-specific activation signals to antigen-specific conventional $CD5^-$ B cells (9, 31, 32). However, the renal function of CD5 antigen on this particular B cell subpopulation remains enigmatic.

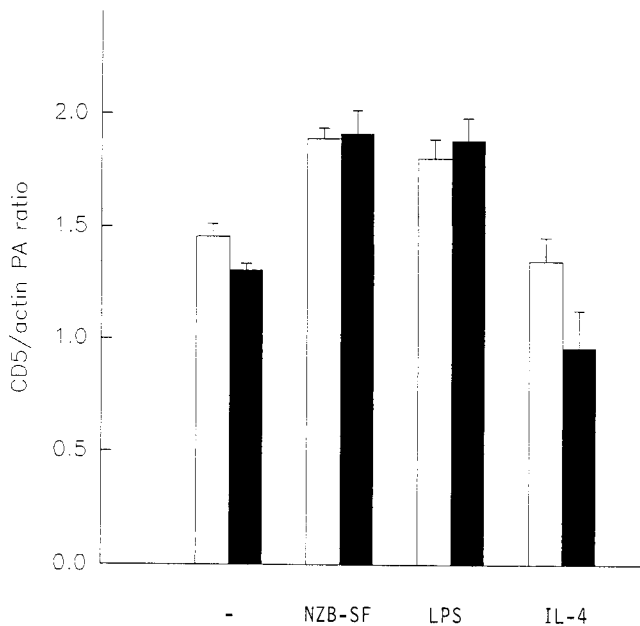

Figure 2. Changes of CD5 mRNA expression by 70Z/3 cells in zinc-deficient culture media with (■) or without (□) supplementation of $ZnCl_2$ (10 μM). The results of autoradiography was scanned by the densitometer and expressed as the ratio of peak absorbance (PA) of each band by CD5 and actin probes. The averages of CD5/actin PA ratio ± SE in three separate experiments were plotted in the figure.

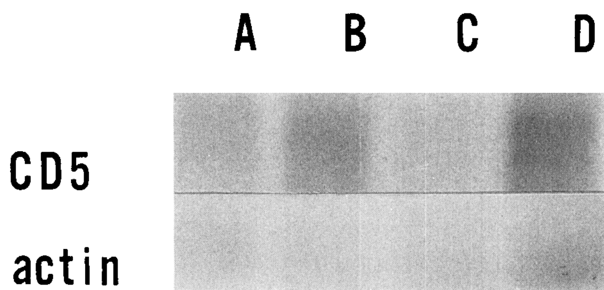

Figure 3. Results of CD5 mRNA expression by 70Z/3 cells by northern blot analysis. 70Z/3 cells were cultured overnight in the presence of IL-4 (10 units/ml) in the regular medium (A), in the zinc-deficient medium (B), and in the zinc-deficient medium supplemented with $ZnCl_2$ (10 μM) (C). Total RNA (5 μg) were then obtained from each group and subjected to northern blot analysis. CD5 mRNA expression by 70Z/3 cells cultured in the regular medium alone were also shown as control (D). The size of CD5 mRNA was 2.2 kb.

Recently, we and other researchers have found that IL-4 can down-regulate CD5 expression on normal as well as malignant B cells both at the level of protein expression and at the level of CD5 mRNA expression (11, 12, 15). In our model system we employed 70Z/3 pre-B leukemia cell line. In this system we also found that CD5 can be up-regulated by exposure to LPS and NZB-SF, a B lineage cell maturation factor we originally isolated from autoimmune-prone NZB mice (13, 14). The expression of sIg by sIg^- 70Z/3 subclones is inducible by activating the transcription of κ light chain on this cell line (33). Several humoral factors, including LPS and IL-1, are known to initiate the transcription of Ig κ light chain by activating NF-κB, one of the DNA-binding proteins, with the structure of a zinc finger (20–23, 33). Therefore, in the process of studies of regulatory mechanism of CD5 expression of this cell line, we decided to study the effect of zinc as well.

For that purpose we depleted zinc from the culture media and studied changes of CD5 expression by 70Z/3 cells. To our surprise, the depletion of zinc rather enhanced the spontaneous as well as induced expression of CD5 by 70Z/3 cells. Other B cell surface markers studied including AA4.1, Ia, B220, and sIg did not change under culture conditions of zinc depletion. Apparent enhanced CD5 expression in zinc-deficient medium alone or in the presence of enhancers (NZB-SF or LPS) returned to the baseline levels when the culture media were supplemented with more than 1 μM $ZnCl_2$ as shown in Results. When 70Z/3 cells were metabolically inactive, zinc did not exert any actions on the CD5 expression which indicates that changes of CD5 expression in zinc-deficient culture conditions are unlikely to be attributable to the changes of binding structures of CD5 molecules to monoclonal Ly-1 antibody. We also found that the actions of LPS and NZB-SF on CD5 expression by 70Z/3 cells were completely abolished in the presence of actinomycin D or cycloheximide in both zinc-deficient medium and in the regular medium (unpublished observations). We reported before that CD5 mRNA levels in 70Z/3 cells were also enhanced in the presence of LPS or NZB-SF (15). Such enhanced expression of CD5 mRNA were also observed in zinc-deficient culture conditions in the presence of these enhancers. However, spontaneous as well as induced CD5 mRNA expression was not significantly increased in culture conditions of zinc deficiency. Taken together, the enhanced expression of CD5 antigen by 70Z/3 cells in zinc-deficient media might be related to a posttranslational event.

In contrast, the down-regulatory effect of IL-4 on CD5 antigen by 70Z/3 cells was significantly depressed under conditions of zinc deficiency. The decrease of CD5 mRNA levels by IL-4 was not clearly observed when 70Z/3 cells were cultured in zinc-deficient medium either. Down-regulatory actions of IL-4 were clearly restored by supplementation of zinc to the culture medium in both RNA and protein levels. The presence of zinc thus seems essential for IL-4 to manifest its down-regulatory action effectively. These results also indicate that IL-4 may exert its down-regulatory action at the level of transcription. A simple explanation of these observations may be that zinc-finger-like DNA-binding protein is involved as a second messenger (as repressor) to transduce the signal elicited by IL-4 in this system. In our preliminary experiments, we also found that actions of IL-4 are abolished by a protein synthesis inhibitor (cycloheximide) and a transcription

inhibitor (actinomycin D) which may also support this hypothesis (unpublished observations). It may be interesting to study the changes of other actions of IL-4 in zinc-depleted culture conditions. However, sIg expression by the 70Z/3 cell subclone employed in this study was not altered in the zinc-deficient culture conditions, although it is well known that sIg can be induced on sIg^- 70Z/3 subclones through the activation of NF-κB (33). One possible explanation is that sIg^+ 70Z/3 cell subclone may not be influenced by NF-κB anymore. In fact we observed previously that sIg^- 70Z/3 cells often became sIg^+ spontaneously and unresponsive to humoral factors in regard to sIg expression (unpublished observation).

In the extensive dialysis steps for preparing zinc-deficient FCS, other components of FCS such as unsaturated fatty acids, small peptides, or amino acids may also have been lost which could contribute to the changes of CD5 expression by 70Z/3 cells. However, the supplementation of $ZnCl_2$ reversed the effects of culture in zinc-deficient media. This finding indicates that the observed changes of CD5 expression are mainly attributable to the depletion of zinc. The supplementation of more than 1.0 μM zinc seemed to be sufficient to reverse the effects of zinc-deficient media on CD5 expression by 70Z/3 cells. Since the physiologic level of zinc is 1 to 2 μM in serum, these observations suggest that even mild deficiency of zinc may change CD5 expression by normal and malignant B cells.

Because of their unique characteristics, $CD5^+$ B cells were often considered to be linked to B cell malignancies as well as to autoimmune diseases (1–4, 9). Conditions which enhance CD5 expression may predispose to development of autoimmune phenomenon and possibly to development of B cell malignancies. This study indicates that zinc deficiency may be one of these conditions. However, it needs to be emphasized that these observations were obtained by using highly artificial *in vitro* culture system employing one particular murine pre-B leukemia cell line. It will be necessary to do similar studies by using other murine and human B cell lines and perhaps B cell populations obtained from normal and autoimmune strains of mice. Then, in the future, the results of such studies can be applied to clinical investigations.

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