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ZINC MODULATES THE MITOGENIC ACTIVATION OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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Summary. - *In the present study we have investigated the effect of Zn salts on the mitogenetic activation of human peripheral blood lymphocytes (PBL). Our results show that Zn^{2+} enhances the level of DNA synthesis in human T lymphocytes stimulated by a mitogenic lectin, phytohemagglutinin (PHA); this effect seems to be mediated through an enhanced expression of both interleukin-2 (IL-2) and transferrin (Trf) receptors. We have also analyzed the mitogenic effect of Zn^{2+} alone on PBL, in the absence of other mitogenic stimuli. In this regard we have identified large light density T lymphocytes as the PBL population which is activated to proliferate by Zn^{2+}. Finally, we showed that Zn^{2+} stimulates natural killer (NK) activity; this effect is apparently not due to a direct action on NK lymphocytes, but is related to endogenous cytokines released by accessory cells which in turn stimulate the cytolytic activity of NK lymphocytes.*

Riassunto (Lo zinco modula la proliferazione dei linfociti umani del sangue periferico). - *Nel presente studio è stato analizzato l'effetto dello Zn sull'attivazione dei linfociti umani del sangue periferico (PBL). I nostri studi hanno indicato che lo Zn^{2+} stimola il livello di sintesi di DNA in linfociti umani stimolati da una lectina mitogenica, la fitoemagglutinina (PHA); questo effetto stimolatorio sembra essere mediato da un aumento dell'espressione dei recettori dell'interleuchina-2 e della transferrina. D'altro canto, noi abbiamo anche analizzato l'azione mitogenica dello Zn^{2+} aggiunto da solo ai PBL in assenza di altri mitogeni. In tal senso noi abbiamo provato che i linfociti T grandi di bassa densità rappresentano la popolazione indotta a proliferare dallo zinco. Infine, si è osservato che lo Zn^{2+} stimola l'attività citotossica di tipo "natural killer" (NK) e tale effetto non sembra dovuto ad una azione diretta dello Zn^{2+} sui linfociti NK, ma alla stimolazione da parte di cellule accessorie del rilascio di citochine endogene che esercitano a loro volta un'azione stimolatoria sull'attività citotossica dei linfociti NK.*

Introduction

General aspects of zinc metabolism

The adult body contains about 2 or 3 g of zinc. High concentrations are found in the choroid of the eye, prostate, liver, bone and hair [1].

Zinc is an essential component of several metalloenzymes. Thus, it is present in many NAD and NADP-linked dehydrogenases, which are enzymes that promote the transfer of hydride ions from substrate molecules to the coenzymes NAD^+ and $NADP^+$. For example, the NAD-requiring enzyme alcohol dehydrogenase of the liver, which catalyzes dehydrogenation of ethanol to yield acetaldehyde, contains two atoms of Zn^{2+}, which appear to bind the NAD^+ coenzyme to the active site of the enzyme. Zinc in the ionic form is also an essential component of DNA and RNA polymerases and thus participates in important enzymatic reactions involved in replication and transcription of genetic information. It is also present in carbonic anhydrase, which catalyzes the hydration of CO_2 to H_2CO_3, and in the proteolytic enzyme carboxypeptidase, secreted into the small intestine. Furthermore, the hormone insulin is stored as a Zn complex. Finally, among the most interesting roles of Zn is the proper functioning of taste and small receptors of the tongue and nasal passages.

Zinc is primarily absorbed by the duodenum and jejunum. Excessive intake of calcium, vitamin D and phytate interferes with its absorption. After absorption, Zn combines loosely with plasma albumin for transport. The normal Zn serum concentration is about 100-140 μg/100 ml. The concentration of Zn in plasma is reduced in a low-Zn diet, in pregnancy and in women on oral contraceptives.

Zinc is excreted primarily by pancreatic and intestinal secretion and finally via the feces. Under normal conditions, urinary excretions range from 450 to 500 μg daily. In burns and following starvation, a significant amount of Zn is excreted in the urine; furthermore, marked losses may

also occur through sweat. The recommended dietary allowance for adults is 15 mg daily. High dietary sources include: sea food, meat/organs, nuts/seeds, dairy products and grains.

Nearly 99 percent of total-body Zn is inside cells, the remainder is in plasma and extracellular fluids. Serum Zn is the source of metal for cellular metabolism. Serum Zn content does not normally vary, but it decreases when intake or absorption is reduced or when urinary losses are increased. Serum Zn also decreases in the acute phase of myocardial infarction, infections, malignancies, hepatitis and other diseases.

Metallothioneine is a small protein which has 60 to 61 residues and contains one-third of its residues as cysteine, avidly binding Zn or Cd, both of which can induce its formation. It is presently believed that one normal role of metallothioneine is to serve as a storage depot for Zn, although it can also function as a detoxification mechanism to prevent heavy ions loading. The protein can bind up to 11% of total metal.

Zinc deficiency in the rat is manifested by retarded growth, alopecia, and lesions in the skin, esophagus and cornea. Hogs fed with processed peanut meal develop a syndrome called parakeratosis, with anorexia, nausea and vomiting. The disease is readily cured by inclusion of 0.02% $ZnCO_3$ in the diet. The disease occurs only if the diet has been supplemented with calcium. Analysis of foodstuffs for Zn are not adequate to reflect their value as dietary sources of this element. Thus soybean, sesame and peanut meals all contain significant amounts of Zn, which seems to be unavailable when ingested by the animals.

Deficiency of Zn in human beings, sometimes associated with calcium and iron deficiency, has been reported in various parts of the Middle East (Iran, Egypt). Endemic Zn deficiency, discovered in some villages in Iran, leads to small stature, anemia, low serum albumin and retarded development of the reproductive system. It is caused by consumption of a traditional local staple of diet, unleavened bread made from partially refined flour. Such bread contains large amounts of phytate, which binds Zn tightly, thus preventing its absorption by the intestine.

In the United States, Zn deficiency has been found in some infants and children, evident by poor growth and appetite, impaired taste acuity (hypogeusia) and low level of Zn in the hair. A variety of disorders in adults have also been reported to cause poor absorption or excessive losses of Zn. These include severe trauma, chronic renal disease, alcoholism, and proteinuria, all of which involve high Zn excretion in the urine.

The most severe clinical disorder caused by Zn deficiency is the rare autosomal, recessive, inherited disease named acrodermatitis enteropathica, which begins in infancy and is manifested by roughness and thick skin, baldness and persistent diarrhea, followed by poor growth and development, infections and sometimes death in a few years. When Zn is administered, clinical remission is rapid and complete with restoration of serum Zn to normal levels.

Zinc and the immune system

It was recently noted [2] that Zn is the nutrient best characterized by its ability to influence immune functions. Zinc deficiency in human and/or experimental animals can result in thymic involution and thymocyte depletion and depression of delayed dermal hypersensitivity (DDH). T lymphocyte numbers, T cell mitogen responses, T-helper function, NK function, and cytotoxic killer-cell activity [3-9].

An example of a human disease associated with unfavourable effects of Zn deficiency on the immune system is represented by sickle cell disease (SCD). In fact Tapazoglou *et al.* provided evidence that the majority of adult SCD patients are Zn-deficient [10]. Furthermore, it was observed that anergy and decreased NK activity in SCD are due to a deficiency of Zn and are reversed by Zn supplementation [10, 11].

Finally, an alteration in T cell subpopulations in SCD [12], and in the response of peripheral blood lymphocytes to T cell mitogens have also been documented; both defects were normalized after *in vivo* Zn administration [13].

Although it is clear that Zn supplementation can enhance immune function in severely Zn-deficient animals and humans, there is evidence suggesting that pharmacologic doses of Zn may be immunostimulatory even in the absence of underlying Zn deficiency [14-16].

However, data on the effects of Zn supplementation on immune functions in subjects without severe Zn deficiency are limited to reports in which small numbers of subjects were studied, diets were usually not evaluated, cellular Zn concentrations were not measured, and/or duration of Zn supplementation was usually limited to one month [14-16]. This period of time (one month) seems too short to allow an adaptation to changes in dietary Zn [17].

Though some of these studies suggest that pharmacologic doses of Zn may be immunostimulatory in elderly people, well-controlled studies with a greater number of subjects are needed, particularly because of the considerable variability in immune function found in elderly people [18]. In this context, regardless of the possible limitations mentioned above, a recent study performed to investigate Zn nutritive and immune functions in 100 healthy elderly subjects who were not receiving Zn supplements is still of some interest [19, 20]. In this population, the incidence of anergy to a panel of seven skin-test antigens was 41%; DDH responses to these antigens were significantly associated with subject plasma Zn concentrations [19]. In addition, subjects with depressed lymphocyte proliferative responses to mitogens had significantly lower platelet Zn concentrations [19].

A large number of *in vitro* studies have provided evidence that Zn is the only ubiquitous cellular component that can function as a lymphocyte mitogen. In fact, it was repeatedly reported that Zn ions activate the proliferation of lymphocytes of different animal species, including humans [21]. This property is displayed only by Zn and

Hg, since other cations including cadmium, copper, silver, chromium, arsenic, beryllium, lanthanum, gold, lead, magnesium, manganese, cobalt and calcium do not activate lymphocytes and many of them are cytotoxins [22]. Thus, Zn stimulates large increases in the levels of ^{3}H-thymidine incorporation between 96 and 144 h of culture, reflecting response kinetics similar to *in vitro* lymphocyte responses to soluble antigens [21]. In contrast, responses to lectin mitogens show optimal human lymphocyte stimulation between 48 and 72 h.

Using sheep erythrocytes to separate T cells and non-T cells in populations of human peripheral leukocytes, Rühl and Kirchner [23] found that Zn is a T cell mitogen. T cell reactivity depends on monocytes and the role of monocytes can be mimicked by a monocyte-derived factor.

Little work has been done so far to determine the subpopulation of T lymphocytes responding to Zn.

Cunningham *et al.* reported that Zn is also a mitogen for B lymphocytes [24]. In this study, however, it was not clarified if T cells were required for the B cell response to occur or if there was a T cell-independent B cell activation as reported for other mitogens. Additional observations showed that adding Zn to cultures of antibody-forming cells from aged mice causes large increases in the specific antibody response [25]. Furthermore, Zn affects the early activation stages of antibody formation and increases the production of IL-1 and IL-4 [26].

Recent studies have reported that Zn stimulates the proliferation of murine thymocytes. Thus, it was shown that Zn enhances the mitogenic response of murine thymocytes to IL-1 or to PHA plus IL-1 [27, 28]. In this context, the observation that Zn allows the thymocytes to respond to IL-1 in the absence of any other mitogenic stimulus is particularly interesting [28].

In this paper we report studies focused on the analysis of the effect of Zn ions on the activation of human lymphocytes with particular emphasis on the study of the effect of this metal on the expression of activation markers (i.e., Trf-IL-2 receptors) and the identification of the lymphocyte subpopulation activated by Zn^{2+}.

Recent achievements

We have studied the effect of Zn^{2+} ions on the activation of PBL with the specific aim of evaluating: a) the lymphocyte subpopulations activated by Zn^{2+} ions in the absence of other mitogens; b) the effect of Zn^{2+} on the expression of activation membrane markers, i.e., Trf and IL-2 receptors, in PHA-stimulated T lymphocytes; c) the effect of Zn^{2+} ions on the antitumor cytotoxic activity of NK lymphocytes.

Effect of Zn^{2+} ions on the activation of human PBMC

We first investigated the effect of Zn^{2+} on the activation of PBMC stimulated with a mitogenic lectin (phytohemagglutinin, PHA). To perform these studies, peripheral blood mononuclear cells (PBMC) were isolated by centrifugation on a Ficoll-Hypaque gradient and then grown at 1×10^6 cells/ml in RPMI 1640 medium containing 5% fetal calf serum (FCS) and 1 μg/ml purified PHA. To some cultures, various concentrations of $ZnCl_2$ ($1\text{-}4 \times 10^{-4}$ M) were added. In control cultures of PBMC, a progressive stimulation of DNA synthesis (as evaluated by ^{3}H-thymidine incorporation) was observed with peak level on day 3 of culture. After this time, a progressive and marked decline of the level of ^{3}H-thymidine incorporation was observed (Fig. 1). The addition of Zn^{2+} ions, at optimal concentrations ($1\text{-}2 \times 10^{-4}$ M), elicited: a) lower levels of DNA synthesis with respect to the control on day 2 of culture; b) a slight stimulation of peak level of DNA synthesis on day 3 of culture; c) at variance with the findings observed in control cultures, the DNA synthesis remained at high levels also in the following days of culture (Fig. 1). These findings were constantly observed on PBMC derived from 10 different healthy donors.

We also analyzed the effect of different Zn^{2+} concentrations on the activation of DNA synthesis in PBMC cultures not stimulated by mitogenic lectins. In the control PBMC culture, a slight activation of DNA synthesis was observed only on day 7 of culture, while Zn^{2+} ions, at optimal concentrations ($1\text{-}2 \times 10^{-4}$ M), elicited a marked activation of DNA synthesis with peak levels on day 7 of culture (Fig. 1).

Similarly, Zn^{2+} enhances the proliferation of T lymphocytes observed in mixed lymphocyte cultures performed by co-culture of two HLA-unrelated subjects.

It is of some interest to underline that $ZnCl_2$ was well tolerated by total PBMC in that up to a concentration of 4×10^{-4} M it did not give rise to any significant cellular

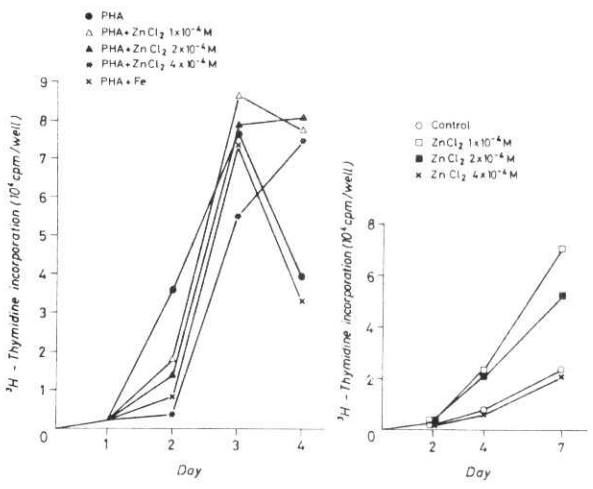

Fig. 1. - Effect of Zn^{2+} on the mitogenic activation of human PBMC. Left panel = PBMC were grown at 1×10^6 cells/ml in the presence of PHA alone or with different concentrations of $ZnCl_2$ (1, 2 and 4×10^{-4}M) or ferric ammonium citrate (10 ng/ml). Right panel = PBMC were grown under standard conditions (control) or in the presence of different concentrations of $ZnCl_2$ as above. For both culture conditions, the level of ^{3}H-thymidine incorporation was measured at each day of culture.

toxicity. At higher concentrations it resulted in a progressive toxicity (data not shown here). However, when the metal was added to cultures of PBMC completely depleted of monocytes (PBL), it gave rise to a strong toxicity at concentrations higher than 1 x 10^{-4} M. This result suggests that monocytes play a key role in the mechanisms protecting the body from the toxic potential related to Zn load; this finding is in agreement with thewell-known function of monocytes in the storage of other metals, such as iron.

Additional experiments were focused on the identification of the lymphocyte subpopulation activated by Zn^{2+} ions. In this regard, PBMC were first depleted of monocytes by two cycles of adherence to Petri dishes and then non-adherent cells (lymphocytes) were separated by centrifugation on a three-step discontinuous gradient of Percoll (d = 1.052; d = 1.061; d = 1.077).

The cells from each fraction were recovered and then grown *in vitro* in the absence or the presence of $ZnCl_2$. Only cells of fraction 1 (d = 1.052) and particularly of fraction 2 (d = 1.061), were induced to proliferate by Zn^{2+} (Fig. 2), while lymphocytes of higher density (fraction 3) were not activated by these ions (Fig. 2). It is interesting to point out that lymphocytes of fraction 2 were stimulated to proliferate even if they were obtained from a lymphocyte population completely depleted of monocytes. This result clearly indicates that the mitogenic response to Zn^{2+} does not require the presence of monocytes. Lymphocytes derived from fraction 2 were composed by NK and large T lymphocytes. To verify the cellular components of fraction 2 which respond to the mitogenic stimulation by Zn^{2+}, we separated these two populations using specific monoclonal antibodies and evaluated their mitogenic response to Zn^{2+}.

These studies clearly indicate that while Zn^{2+} stimulates the proliferation of the total light lymphocyte population, it is unable to induce the proliferation of purified NK lymphocytes (Table 1). This result clearly shows that the lymphocyte subpopulation responsive to Zn^{2+} is composed of large light T lymphocytes. These cells may represent *in vivo* spontaneously activated T cells and memory T cells. It is interesting to note that, at variance with Zn^{2+}, IL-2 is capable of stimulating DNA synthesis of both light density T lymphocytes and NK cells.

Effect of zinc ions on the expression of Trf and IL-2 receptors in PHA-stimulated T lymphocytes

Previous studies have provided evidence that in mitogen-activated T lymphocytes, first the expression of IL-2 receptors is activated followed by that of Trf receptors [29]; both membrane structures are essential for cell cycle progression of T lymphocytes. To investigate the effect of Zn^{2+} ions on the expression of both IL-2 and Trf receptors, we have grown PHA-stimulated PBMC in the absence or in the presence of increasing concentrations of $ZnCl_2$ (from 1 to 4 x 10^{-4} M) and the level of ^{125}I-IL-2 and ^{125}I-Trf binding was then evaluated each day of culture. In control cultures the expression of Trf receptors is progressively

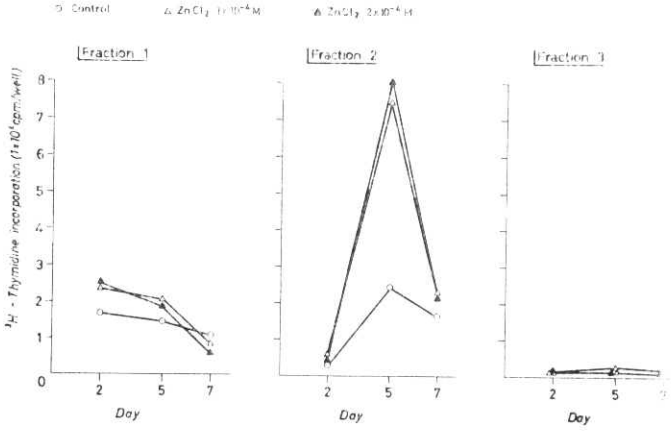

Fig. 2. - Effect of Zn^{2+} on the activation of human PBL separated according to density by centrifugation on a discontinuous gradient of Percoll. The density of lymphocytes was the following: fraction 1, d = 1.052; fraction 2, d = 1.064; fraction 3, d = 1.077.

Table 1. - *^{3}H-thymidine incorporation of total light density lymphocytes (d = 1.062) and purified NK lymphocytes grown either in the absence or in the presence of $ZnCl_2$*

Cell population 1 x 10^3 cpm/well	^{3}H-thymidine incorporation Day 1	Day 3	Day 6	Day 8
Total, control	1.4	8.6	14.2	8.9
Total + Zn^{2+} 1 x 10^{-4} M	1.8	14.5	41.0	26.2
Purified NK, control	7	27	46.5	37.1
Purified NK + Zn 1 x 10^{-4} M	4.4	24	51.5	44.3

activated, reaching peak expression on day 3 and sharply declining thereafter (Fig. 3). In cells grown in the presence of optimal $ZnCl_2$ concentrations (1-2 x 10^{-4} M) a similar initial kinetics of Trf receptor expression was observed, with peak levels of expression observed at day 3 of culture. However, the peak level of expression observed in cultures grown in the presence of Zn^{2+} ions is significantly higher than that observed in control cultures. Furthermore, in Zn^{2+}-treated cultures, in the last days of culture (after day 3), the level of Trf receptor expression remained at peak levels and did not decline as observed in control cultures (Fig. 3). Scatchard analysis of ^{125}I-Trf binding data showed the following: a) the enhancement of Trf receptor expression induced by Zn^{2+} is due to an increase in the number of receptors rather than to a modification of their affinity for the ligand; b) Zn^{2+} ions induced a dose-dependent increase in the number of Trf receptors (Fig. 3). For comparison, the effect of iron on the expression of Trf receptors in mitogen activated T lymphocytes is shown (Fig. 3). As previously reported [30], iron salts elicited a marked reduction in the number of Trf receptors (Fig. 3). Furthermore, other elements including copper, selenium and lithium did not affect the expression of Trf receptors in PHA-stimulated

lymphocytes. Thus, it seems evident that the expression of Trf receptors in mitogen-activated T lymphocytes is controlled by the intracellular concentration of iron and Zn through a balance mechanism in that an increase in iron supplementation induces a decrease in the expression of the receptor, while an opposite effect is elicited by a rise of Zn supply for the cells.

Parallel experiments were performed to investigate a possible effect of $ZnCl_2$ supplementation on the expression of IL-2 receptors. These studies showed that the level of ^{125}IL-2 binding was similar in both Zn supplemented and control cultures, the only exception being that in the former the level of binding remained high after peak expression while in the latter a sharp decline was observed after peak expression (Table 2). Furthermore, we also observed that Zn^{2+} supplementation to PHA-stimulated PBMC elicited an enhancement of interferon-γ synthesis and release (Fig. 4).

Effect of Zn^{2+} ions on NK activity

In another set of experiments we evaluated the effect of Zn^{2+} on the antitumor cytotoxic activity of NK lymphocytes. A first set of experiments was performed by incubating PBMC for 4 days in the presence of 1 or 2 x 10^{-4} M Zn^{2+} and then evaluating the level of NK activity by a standard 4 h 51-chromium release assay using erythroleukemia K562 cells. Cells grown in the presence of Zn^{2+} ions exhibited a higher level of NK cytotoxic activity than controls (Fig. 5); maximal cytotoxic activity was observed for cells grown with 1 x 10^{-4} M Zn^{2+}. In fact, the lytic activity of PBMC grown in the presence of Zn^{2+} ions doubled as compared to the values observed for controls. However, Zn^{2+} ions did not induce lymphokine activated killer (LAK) activity, i.e., the capacity to lyse NK-resistant tumor cells.

In another set of experiments, NK lymphocytes were purified as described above and their cytolytic activity evaluated after culture in standard conditions and in the presence of 1 x 10^{-4} M Zn^{2+}. These experiments showed that Zn^{2+} is unable to enhance the NK activity of purified NK lymphocytes. This result strongly suggests that the stimulatory effect of Zn^{2+} ions on NK activity observed in PBMC cultures is not related to a direct action of this metal on NK lymphocytes, but is probably related to the release of endogenous cytokines which exert a stimulatory effect on NK activity.

Table 2. - *Effect of Zn^{2+} on the expression of IL-2 receptors in PHA-stimulated human PBMC*

Cell population	(^{125}I) IL-2 bound 1 x 10^3 cpm/ 2 x 10^6 cells			
	Day 1	Day 2	Day 3	Day 4
Control	1.5	2.3	5.7	3.6
Zn^{2+} 1 x 10^{-4} M	1.8	1.6	4.3	7.6
Zn^{2+} 2 x 10^{-4} M	1.8	1.8	4.7	6.2
Zn^{2+} 3 x 10^{-4} M	1.7	1.7	5	5.7

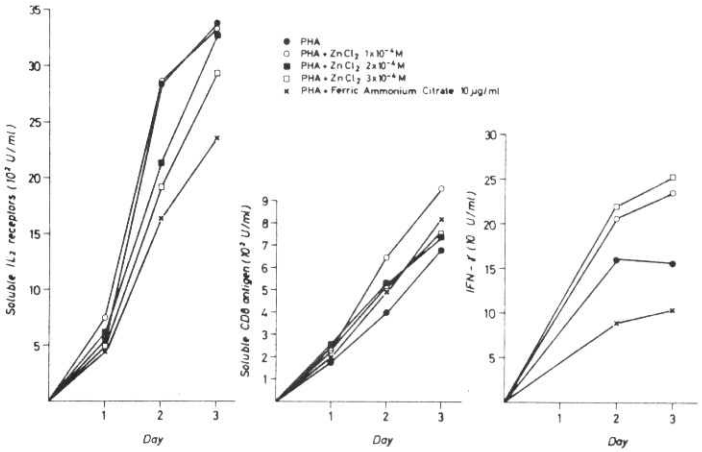

Fig. 4. - Effect of Zn^{2+} on the release of soluble IL-2 receptors, soluble CD8 antigen and IFN-γ by PHA-stimulated PBMC. The concentrations of these three markers in the culture supernatants were evaluated by a sensitive immuno-enzymatic method.

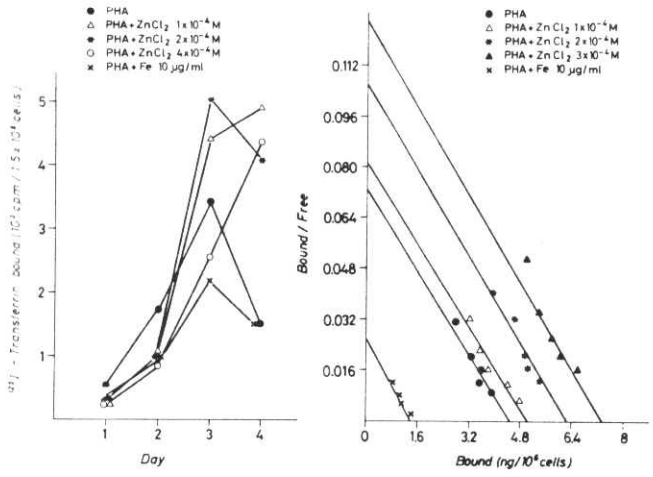

Fig. 3. - Effect of Zn^{2+} on the expression of Trf receptors in PHA-stimulated PBMC grown in the absence or in the presence of Zn^{2+}. Left panel = At each day of culture the cells were harvested and their Trf binding capacity was evaluated by incubation in the presence of ^{125}I-Trf, under the experimental conditions previously reported [38]; Right panel = Scatchard analysis of ^{125}I-Trf binding data to PHA-stimulated PBMC grown for 4 days; to perform such an analysis, cells were incubated in the presence of a fixed amount of ^{125}I-Trf (500 ng/ml) and increasing concentrations of cold Trf (0-500 ng/ml).

Discussion

The studies described above shed some light on the mechanisms responsible for the stimulation of lymphocyte activation induced by Zn^{2+} ions. Initially, our experiments were focused on analyzing the effect of different

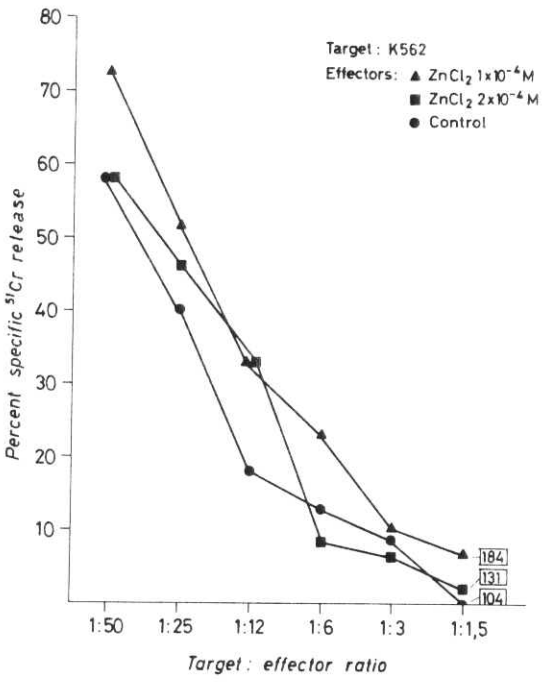

Fig. 5. - Effect of Zn^{2+} ions on cell-mediated cytotoxic activity of PBMC. PBMC were grown for 48 h either in the absence or in the presence of Zn^{2+} ions and their cytotoxic activity against a NK-sensitive cell target (K562) was measured by means of a standard 4 h-^{51}Cr-release assay.

degrees of Zn^{2+} loading on the expression of some activation markers, including Trf and IL-2 receptors. The results of these experiments showed that Zn^{2+} ions elicited a slight, but significant, stimulation of both DNA synthesis and Trf and IL-2 receptors expression in PHA-stimulated PBMC cultures. More significantly, while in control cells the expression of these markers rapidly declines after a peak level of expression is reached, in Zn^{2+}-supplemented cultures, the expression of the above mentioned activation markers does not decline after peak expression is reached. This sustained expression of activation markers does not seem to be related to a block in cell cycle progression elicited by Zn^{2+} ions, since Zn^{2+}-supplemented lymphocyte cultures are equally or even more capable of mitogenic division than control ones.

The stimulation of Trf receptor expression induced by Zn^{2+} ions is of particular interest. In fact, previous studies have shown that iron down modulates the expression of Trf receptors [30]; this effect is largely due to a modulation of the level of Trf receptor mRNA induced by iron. On the other hand, here we show that also Zn^{2+} ions stimulate the expression of this receptor.

Further studies are required to evaluate the molecular mechanisms responsible for the upregulation of Trf receptor expression induced by Zn^{2+} ions.

Altogether these findings suggest that the level of Trf receptor is modulated through a balance between the opposite effects elicited by the intracellular concentration of Fe and Zn. Furthermore, since previous studies have shown that PHA stimulates the uptake of Trf-bound Zn by human lymphocytes [31] it is reasonable to postulate that the rise of intracellular Zn may represent one of the factors responsible for the stimulation of Trf receptors expression observed during T-lymphocyte mitogenesis. Future studies will be focused on investigating whether in PHA-stimulated T lymphocytes the stimulation of Trf receptor expression elicited by Zn^{2+} ions is related to the stimulation of IL-2 synthesis. In fact, previous studies have shown that in lectin-activated T lymphocytes, exogenous IL-2 is a major modulator of the expression of the Trf receptor gene [32, 33].

Our second set of experiments was focused on identifying the lymphocyte subpopulation which is activated by Zn^{2+} ions. These studies provided evidence that Zn^{2+} ions stimulate DNA synthesis of light density T lymphocytes co-purifying with NK lymphocytes. In contrast, purified NK lymphocytes were not stimulated by Zn^{2+} ions. It is interesting that IL-2, at variance with Zn^{2+} ions, stimulates DNA synthesis of both light-density T lymphocytes and NK cells [34, 35]. Light density lymphocytes activated by IL-2 were defined as spontaneously proliferating IL-2 lymphocytes [35, 36] and it is conceivable that these same lymphocytes were also activated by Zn^{2+} ions.

Light density T lymphocytes activated by Zn^{2+} ions may represent either immature T cell precursors or activated T cells in the course of an immune response.

Finally, Zn^{2+} ions stimulate the cytotoxic activity of NK lymphocytes. However, this effect was observed only when Zn^{2+} ions were added to PBMC, but not to purified NK lymphocytes. This finding clearly supports the hypothesis that the stimulatory action of Zn^{2+} ions on NK activity is indirect. In the regard, this stimulatory activity of Zn^{2+} ions may be related to the recently described capacity of Zn^{2+} ions to induce the synthesis and release of interferon [37], which is known to enhance NK activity. The stimulatory activity of Zn^{2+} ions on IFN-γ synthesis and release have also been confirmed by our studies, reported here.

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