Zinc-Mediated Inhibition of Cyclic Nucleotide Phosphodiesterase Activity and Expression Suppresses TNF-α and IL-1β Production in Monocytes by Elevation of Guanosine 3′,5′-Cyclic Monophosphate

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Zinc-Mediated Inhibition of Cyclic Nucleotide Phosphodiesterase Activity and Expression Suppresses TNF-α and IL-1β Production in Monocytes by Elevation of Guanosine 3′,5′-Cyclic Monophosphate

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The trace element zinc affects several aspects of immune function, such as the release of proinflammatory cytokines from monocytes. We investigated the role of cyclic nucleotide signaling in zinc inhibition of LPS-induced TNF-α and IL-1β release from primary human monocytes and the monocytic cell line Mono Mac1. Zinc reversibly inhibited enzyme activity of phosphodiesterase-1 (PDE-1), PDE-3, and PDE-4 in cellular lysate. It additionally reduced mRNA expression of PDE-1C, PDE-4A, and PDE-4B in intact cells. Although these PDE can also hydrolyze cAMP, only the cellular level of cGMP was increased after incubation with zinc, whereas CAMP was found to be even slightly reduced due to inhibition of its synthesis. To investigate whether an increase in cGMP alone is sufficient to inhibit cytokine release, the cGMP analogues 8-bromo-cGMP and dibutyryl cGMP as well as the NO donor 5-nitrosocysteine were used. All three treatments inhibited TNF-α and IL-1β release after stimulation with LPS. Inhibition of soluble guanylate cyclase-mediated cGMP synthesis with LY83583 reversed the inhibitory effect of zinc on LPS-induced cytokine release. In conclusion, inhibition of PDE by zinc abrogates the LPS-induced release of TNF-α and IL-1β by increasing intracellular cGMP levels.

Zinc is a trace element that affects many aspects of immune function, including thymic development and the activity of T cells, B cells, and NK cells (1). In particular, zinc has contradictory effects on monocyte activity and the secretion of proinflammatory cytokines. On the one hand, it was shown that zinc stimulates release of the monokines IL-6, IL-1β, and TNF-α in human PBMC (2) and induces mRNA expression of TNF-α (3). On the other hand, zinc negatively regulates TNF-α, IL-8, and IL-1β gene expression in the HL-60 monocyte/macrophage cell line (4). Furthermore, zinc acts synergistically with LPS if applied simultaneously in substimulatory concentrations (5), but zinc pretreatment protects against LPS-induced hepatotoxicity by attenuation of TNF-α production in liver tissue during liver injury in mice (6).

Inhibition of TNF-α production can be mediated by cyclic nucleotide signaling. The intracellular level of cyclic nucleotides is regulated by their synthesis by adenylate (AC) and guanylate cyclases and their hydrolysis into the corresponding inactive 5′-nucleotide monophosphates by phosphodiesterases (PDE). Numerous studies show that selective PDE-4 inhibitors, such as rolipram and Ro-20-1724, attenuate TNF-α production in human monocytes and PBMC by elevation of the intracellular cAMP concentration (7–11). The cGMP analogues, dibutyryl cGMP (DB-cGMP) and 8-bromo-cGMP (Br-cGMP) were found to attenuate LPS-induced TNF-α secretion in primary monocytes and murine bone marrow-derived macrophages (8, 12). Eleven distinct families of PDE (PDE-1 to -11) have been identified, most of which include more than one subtype. To date, PDE-1, which hydrolyzes both cGMP and cAMP, cAMP-selective PDE-3, and cAMP-specific PDE-4 have been observed in human monocytes (13, 14). Although an effect of zinc on cyclic nucleotide metabolism in monocytes has not been investigated, zinc inhibits several isoforms of purified recombinant PDE that are present in these cells, including PDE-4A and PDE-3 (15, 16). In PC12 pheochromocytoma cells, an involvement of zinc in cyclic nucleotide metabolism was demonstrated. Zinc treatment of PC12 led to an inhibition of cGMP hydrolysis and caused an elevation of the intracellular cGMP content (17).

The aim of this study was to investigate whether zinc suppresses cytokine release of monocytes via an effect on cyclic nucleotide metabolism, especially by modulating PDE activity. We show that zinc has a dual effect, regulating cellular PDE activity as a direct inhibitor of the catalytic activity of all isozymes of phosphodiesterases expressed in monocytes and additionally as an inhibitor of PDE gene expression. Moreover, zinc-induced inhibition of PDE elevates intracellular cGMP, but not cAMP, and suppresses the LPS-induced production of TNF-α and IL-1β.

Materials and Methods

**Media and reagents**

RPMI 1640 medium, sodium pyruvate, L-glutamine, nonessential amino acids, penicillin, streptomycin, and PBS were purchased from Cambrex. Ficoll was obtained from Biochrom, and FCS was purchased from PAA.
Laboratories. Chlorpromazine, 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro-20-1724), and quazinone were purchased from Calbiochem, and 8-Br-cGMP, DB-cGMP, cAMP, and cGMP were purchased from BioLog LSI. Isobutyl-1-methylxanthine (IBMX), forskolin (FSK), LPS Escherichia coli serotype 0111:B4, 6-(phenylamo)-5,8-quinolinolenedione (LY83583), sodium pyrophosphate, sodium nitrite, t-cysteine hydrochloride, potassium iodide, N,N',N'-tetrakis (2-pyridylmethyl)- ethylenediamine (TFEN) were obtained from Sigma-Aldrich. Directly before incubation, a 100 mM stock solution of the NO donor 5-nitroso-cysteine (SNOC) was prepared from t-cysteine hydrochloride and sodium nitrite as previously described (18). All other reagents were purchased from standard sources.

Cell culture

All cell lines were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen and cultured at 37°C in a humidified 5% CO2 atmosphere in RPMI 1640 medium containing 10% FCS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. The human histiocytic lymphoma cell line U937 was cultured directly in this medium. Media for the human acute monocytic leukemia cell lines THP-1 and Mono Mac1 were additionally supplemented with 5 μL/2-ME (THP-1) or with nonessential amino acids and 1 mM sodium pyruvate (Mono Mac1).

Isolation and culture of PBMC and monocytes

PBMC were isolated from heparinized peripheral venous blood from healthy donors by centrifugation over Ficoll-Hypaque, washed three times with PBS, and resuspended in RPMI 1640 medium (containing 10% heat-inactivated FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine). For enrichment of monocytes, PBMC were seeded in 100-mm diameter plastic culture dishes at a density of 2×10^6 cells/ml and were incubated for 1 h at 37°C in 5% CO2. Nonadherent cells were removed by washing with medium at 37°C. The monocyte preparations contained 80% CD14+ cells, as determined by flow cytometry.

Measurement of cellular cAMP

For analysis of cellular cAMP, 4×10^6 Mono Mac1 cells were seeded in 24-well tissue culture dishes for 1 h (PBMC/monocytes) or in six-well tissue culture dishes overnight (Mono Mac1). After the incubations, culture supernatants were harvested and stored at −80°C. For measurement of cellular cytokine content, cells were washed twice with ice-cold PBS; suspended in 50 mM Tris-HCl, pH 7.4, containing 1% (w/v) Triton X-100, 300 mM NaCl, 5 mM EDTA, and 0.02% (w/v) sodium azide; incubated on ice for 15 min; lysed by sonification. The assay was performed according to the manufacturers’ instructions. 

RT-PCR

For investigation of PDE subtype expression, Mono Mac1, U937, and THP-1 were cultured routinely in 25-cm² plastic flasks. RNA of PBMC and monocytes was extracted 1 h after isolation. For the investigation of changes in PDE mRNA expression, Mono Mac1 cells (3×10^6/ml) were cultured in six-well tissue culture dishes overnight before treatment with zine and/or LPS. For all samples, total RNA was isolated using the Rneasy kit (Qiagen), and cDNA was prepared using the Reverse Transcription System (Promega) according to the manufacturers’ instructions. PCR was performed using HotStarTag DNA polymerase (Qiagen) in a final volume of 50 μL using 50 ng/μL cDNA with the following cycle parameters: 95°C for 15 min, 35 cycles of denaturation at 95°C for 40 s, annealing at 60°C for 1 min, and extension at 72°C for 3 min, with a 5-min extension at the last cycle. β-Actin was used as a housekeeping gene. In the case of PDE-1A, -1B, -4, -6A, and -6B, 31 cycles of amplification were used. TNF-α gene products were amplified in 28 cycles. Primers (TIB Molbiol) were added at a final concentration of 1 μM (PDE), 0.8 μM (TNF-α), 0.15 μM (IL-1β), and 0.1 μM (β-actin).

Determination of PDE activity by HPLC

To measure PDE activity, 1×10^6 Mono Mac1 cells were harvested in sample buffer (25 mM HEPES, 5 mM glucose, 120 mM NaCl, 5.4 mM KCl, 1.3 mM CaCl2, 1 mM MgCl2, and 1 mM Na2HPO4, pH 7.35) and lysed by sonication. The assay was initiated by addition of cGMP or cAMP (final concentration, 50 μM), and incubated for 1 h at 37°C. At the end of the reactions, the remaining cyclic nucleotide concentrations were measured in at least 10-fold excess of the K_M of the PDE (see Table 1) using the maximum reaction velocity throughout the entire experiment. The reaction was stopped by boiling for 5 min, and any precipitate was removed by centrifugation at 7000×g for 5 min. Cyclic nucleotides and 5'-mononucleotides were separated by HPLC on a Eurosil C18 column (Knauer) using 25 mM triethylammonium formate, pH 6.5, as an eluent, containing 8 or 4.5% methanol for cAMP or cGMP, respectively. Absorption was measured with an MDS-5020 UV detector (Knauer) at 258 nm (cAMP) or 252 nm (cGMP). Data were acquired and processed with Eurochrom 2000 chromatography software (Knauer), and PDE activity was calculated as the decrease in peak area for the cyclic nucleotide. The concentration of endogenous cyclic nucleotides in the lysate was too low to be detected with this method and can be disregarded.

Measurement of intracellular cAMP

For analysis of intracellular cAMP, 4×10^6 Mono Mac1 cells were seeded in polypropylene tubes in 1 ml of culture medium. After incubation at 37°C and 5% CO2, the medium was removed by centrifugation at 5 min for 7000×g. The cellular cAMP level was measured using the Delfia cAMP kit (PerkinElmer), a time-resolved fluoroimmunoassay based on europium dissociative fluorescence enhancement. The assay was performed according to the manufacturers’ instructions with some modifications. Samples were treated with 4 mM IBMX immediately before addition of Delfia lysis buffer and sample buffer and were incubated for 10 min at room temperature. The cAMP assay plate was incubated with anti-cAMP serum for 3 min. Anti-cAMP serum was removed with three washing steps using Delfia wash buffer. Fifty microliters of standards and samples were added to the plate and incubated for 30 min with gentle shaking. The Eu-cAMP tracer solution was added and incubated for an additional 30 min. After four washes, enhancement solution was added and incubated for 5 min at room temperature. The plates were read using a Tecan Ultra 384 fluorometer with the following setup: 340-nm excitation filter; 612-nm emission filter; counting delay, 400 μs; and counting window, 400 μs.

Measurement of intracellular cGMP

For determination of cellular cGMP, Mono Mac1 cells (2.5×10^6 in 1 ml of medium) were seeded in polypropylene tubes and incubated for 6 h at 37°C in 5% CO2. Afterward, cells were pelleted by centrifugation at 3000×g and 4°C for 5 min and subjected to a purification procedure as previously reported (23). Briefly, cells were lysed by addition of 200 μL of EDTA (30 mM) and boiled for 5 min. Proteins were precipitated by addition of 800 μL of ice-cold ethanol, homogenized for 1 min, and incubated for 30 min at 4°C. Proteins and cell debris were removed by centrifugation at 3000×g and 4°C for 10 min. The supernatant was evaporated under a nitrogen flow at 56°C, and cGMP in the residue was determined using a cGMP enzyme immunoassay (IBL Hamburg) based on the competition of free cGMP and cGMP bound to acetylicholinesterase, according to the manufacturers’ instructions.

Statistical analysis

Statistical significance of experimental results was calculated by Student’s t test.

INHIBITION OF PDE ACTIVITY BY ZINC

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Results

PDE expression in monocyctic cell lines and primary cells

Monocytes express three isozymes of PDE: cAMP- and cGMP-hydrolyzing PDE-1 and cAMP-degrading isozymes 3 and 4 (13, 14). The expression profiles of the subtypes of these PDE were analyzed by RT-PCR and compared in human PBMC, primary monocytes, and three monocyctic cell lines (Table I). While PDE-1A, PDE-3A, and PDE-4C mRNA were not detected in any cell line or in primary cells, mRNA encoding for PDE-1B, PDE-1C, PDE-3B, PDE-4A, PDE-4B, and PDE-4D were found in PBMC, monocytes, and Mono Mac1 cells. The expression profiles of the remaining two cell lines were similar, except that U937 cells lacked both PDE-3B and PDE-4A, and THP-1 cells did not express PDE-1C. In accordance with these results, the mature monocyctic cell line Mono Mac1 was chosen as a model for studying the effect of zinc on PDE activity in monocytes.

Concentration-dependent effect of zinc ions on TNF-α release

To establish appropriate conditions for investigation of the inhibitory effect of zinc on LPS-induced secretion of proinflammatory monokines, the release of TNF-α from Mono Mac1 into the supernatant was quantified by ELISA (Fig. 1A). Cells were stimulated with LPS (250 ng/ml) in the presence of zinc concentrations ranging from 1 to 25 μM together with pyrithione (50 μM), an ionophore that facilitates zinc uptake. Zinc has differential effects on TNF-α release depending on its concentration (Fig. 1A). Although 1 μM zinc in the presence of pyrithione increased TNF-α production significantly, 25 μM suppressed the secretion of this cytokine. To elucidate whether the inhibitory effect of zinc depends on the presence of pyrithione, primary monocytes were treated with LPS and zinc without the ionophore. Although higher concentrations were required, zinc inhibited TNF-α release in the absence of pyrithione (Fig. 1B). To ensure that the reduction in TNF-α synthesis was not mediated by cytotoxic effects of zinc, membrane integrity was measured with propidium iodide staining, which showed that none of the zinc concentrations used in this paper was toxic (Fig. 1C).

Inhibition of PDE by zinc ions in cellular lysate

Zinc affects cGMP signaling via inhibition of PDE (17). We investigated whether this effect is specific for cGMP-degrading PDE isozymes or whether zinc acts as a general PDE inhibitor, which also affects the hydrolysis of cAMP. Using selective inhibitors for PDE-1 (chlorpromazine), PDE-3 (quazinone), and PDE-4 (Ro-20-2417), the activity of each isozyme was measured separately in the presence of inhibitors for the other two. To this end, 50 μM cyclic nucleotides were added to Mono Mac1 lysate, and degradation into 5′-monophosphate was monitored by HPLC. The isozymes contribute to the hydrolysis of cAMP in the order PDE-1 > PDE-3 > PDE-4 (Fig. 2A). Hydrolysis of cAMP by all three isozymes was completely inhibited by incubation of the lysate with 50 μM ZnSO4 30 min before the addition of cAMP. As shown in Fig. 2C, preincubation with zinc ions inhibited PDE-1-mediated cGMP hydrolysis completely, whereas PDE-3 and PDE-4 did not contribute to cGMP degradation. Additionally, the application of all three inhibitors blocked cAMP (Fig. 2B) and cGMP (Fig. 2D) degradation, indicating that there is no noteworthy contribution of other PDE isoforms.

Table I. Expression profile of cyclic nucleotide PDE subtypes in monocyctic cell lines, PBMC, and primary monocytes

<table>
<thead>
<tr>
<th>Subtype</th>
<th>PBMC</th>
<th>Monocytes</th>
<th>U937</th>
<th>THP-1</th>
<th>Mono Mac1</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDE-1A</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>PDE-1B</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PDE-1C</td>
<td>(+)</td>
<td>(+)</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>PDE-3A</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>PDE-3B</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PDE-3A</td>
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<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
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</table>

*+, expression of subtype detected; −, expression of subtype not detected; (+), weak expression of subtype detected. RT-PCR was used for the detection of mRNA for the subtypes of PDE-1, PDE-3, and PDE-4. Human prostate cDNA, which expresses all subtypes of PDE-1 and −4, was used as a positive control. Human colon cDNA served as a positive control for PDE-3A (19). Data are representative of four independent experiments.
Reversibility of PDE inhibition by zinc

To study the reversibility of the zinc inhibition of PDE, lysate of Mono Mac1 cells was incubated with 50 μM ZnSO₄ for 1 h, resulting in an inhibition of both cAMP and cGMP hydrolysis that was partially reversible with addition of TPEN to the lysate (Fig. 4A). Upon addition of zinc, cAMP and cGMP hydrolysis was reversed. In contrast, incubation of intact Mono Mac1 cells with 25 μM ZnSO₄ plus 50 μM pyrithione for 1 h resulted in decreased cAMP and cGMP hydrolysis that was only partially reversible by addition of TPEN to the lysate (Fig. 4B), indicating a dual effect of zinc on enzyme activity as well as expression of PDE. Therefore, the expression of PDE isozymes was investigated by RT-PCR. After 6 h of zinc treatment, down-regulation of PDE-1B and PDE-4D and did not affect PDE-3B mRNA expression. These latter PDE isozymes might still be expressed and contribute to the remaining PDE activity of ~30%.

Zinc suppresses LPS-induced up-regulation of PDE-4B expression

In human monocytes the expression of PDE-4B is increased by LPS stimulation (24). Therefore, the role of zinc inhibition in PDE activity and expression was investigated as a possible mechanism to inhibit LPS-induced signals. Treatment of Mono Mac1 cells with LPS resulted in up-regulation of PDE-4B mRNA (Fig. 5A) and significantly increased cAMP hydrolysis (Fig. 5B). In accordance with the fact that cGMP is not a substrate for PDE-4, no significant effect of LPS on cGMP degradation was observed (Fig. 5B). Coincubation with zinc and pyrithione eliminated the LPS-induced up-regulation of PDE-4B mRNA expression (Fig. 5A) and reduced the hydrolysis of cAMP and cGMP to levels comparable to those observed with zinc and pyrithione alone (Fig. 5B).

Down-regulation of LPS-induced release of TNF-α and IL-1β by zinc ions

Next, the effects of zinc or the unspecific PDE inhibitor, IBMX, on the induction of IL-1β and TNF-α mRNA expression were investigated in Mono Mac1 cells (Fig. 6A). LPS-induced TNF-α and IL-1β mRNA expressions were blocked by addition of zinc (25 μM) plus pyrithione (50 μM) as well as by preincubation with IBMX (4 mM). Accordingly, when IL-1β and TNF-α were measured by ELISA in the supernatant of PBMC, the application of zinc plus pyrithione as well as the unspecific inhibition of PDE by IBMX blocked LPS-induced release of both cytokines (Fig. 6B).

To investigate a possible effect of zinc on the secretory level, TNF-α and IL-1β were measured in supernatant and lysate of primary monocytes after treatment with LPS in the presence or the absence of zinc (Fig. 6C). Zinc abrogated the stimulatory effect of LPS in lysate and supernatant equally, with no zinc-induced accumulation of cytokines in the cellular fraction. Taken together, the data indicate that zinc acts via regulation of the mRNA levels of TNF-α and IL-1β.

Zinc inhibition of PDE elevates intracellular cGMP, but not cAMP

Zinc inhibition of PDE should lead to an elevation of intracellular cyclic nucleotides. However, an increase in cellular cAMP could...
FIGURE 4. Effects of zinc ions on PDE activity and mRNA expression. The degradation of cAMP or cGMP was measured by HPLC in the lysate of Mono Mac1 cells (A and B). The values represent the mean ± SEM from at least three independent measurements. Statistically significant (p < 0.05) PDE activity changes are indicated (†). A, Lysate was treated with ZnSO4 (50 μM) for 30 min before addition of cyclic nucleotides (zinc). To assess the reversibility of this incubation, zinc-treated lysate was also incubated with 250 μM of the chelator TPEN for 1 h (TPEN). B, Before lysis, Mono Mac1 cells were incubated for 24 h in the absence or the presence of 25 μM ZnSO4 and 50 μM pyrithione (24h zinc). Lysate of zinc-treated cells was incubated with 250 μM TPEN for 1 h before addition of cyclic nucleotides (TPEN). C, Cells were incubated in the absence or the presence of ZnSO4 (25 μM) plus pyrithione (50 μM) for 6 h. The mRNA expression of PDE subtypes was determined by RT-PCR. Data are representative of three independent experiments.

not be observed when Mono Mac1 cells were treated with zinc (25 μM) and pyrithione (50 μM) for 0.5–6 h (data not shown). Conversely, zinc/pyrithione treatment seemed to have an inhibitory effect on the formation of cAMP, because it significantly reduced the cellular basal level of cAMP and inhibited the increase in cAMP induced by treatment with IBMX together with the AC activator FSK (Fig. 7A). In contrast, incubation of Mono Mac1 cells with zinc plus pyrithione resulted in an elevation of the intracellular cGMP concentration in untreated cells and in the presence of LPS (Fig. 7B). Despite the ability of zinc to inhibit the catalytic activity of all PDE isozymes expressed in Mono Mac1 cells, it increased only the level of cellular cGMP, not that of cAMP.

Role of cGMP in zinc inhibition of TNF-α and IL-1β release

We investigated whether an increase in cellular cGMP can mediate zinc inhibition of LPS-induced cytokine secretion. LPS-induced TNF-α and IL-1β secretion from PBMC was inhibited by two membrane-permeant cGMP analogues, 8-Br-cGMP and DB-cGMP, in a dose-dependent manner (Fig. 8, A and C). To activate cGMP synthesis by soluble guanylate cyclase (sGC), the NO donor SNOC was used. SNOC administration in the presence of LPS resulted in inhibition of TNF-α and IL-1β release from human monocytes (Fig. 8, B and D).

The fact that cGMP inhibits LPS-induced TNF-α and IL-1β release does not necessarily mean that the inhibitory effect of zinc involves cGMP. Therefore, LY83583, an inhibitor of sGC, was used to inhibit cGMP synthesis in primary monocytes. LPS-induced TNF-α release was not affected by LY83583 (Fig. 9A). However, the effect of 1 μM zinc in the presence of pyrithione, a concentration that suppresses LPS-induced TNF-α release by >90% in human monocytes, was significantly antagonized by pre-incubation with LY83583, confirming that cGMP is required for the inhibitory effect of zinc on TNF-α release. In contrast to TNF-α, LPS-induced release of IL-1β was increased in the presence of LY83583. Because the concentration of cGMP did not increase during stimulation with LPS (Fig. 7B), basal levels of cellular cGMP seem to inhibit the release of this cytokine. Nevertheless, zinc-mediated suppression of LPS-induced IL-1β release was also reversed by LY83583 (Fig. 9B), confirming a role for cGMP in the effect of zinc on both cytokines.

Discussion

Zinc supplementation has seemingly contradictory effects on LPS-induced production of proinflammatory cytokines. It stimulates the release of the monokines IL-6, IL-1β, and TNF-α in human PBMC (2); induces TNF-α mRNA expression (3); and acts synergistically with LPS if applied simultaneously in substimulatory concentrations (5). In contrast, zinc was found to negatively regulate TNF-α, IL-8, and IL-1β gene expression in the HL-60 monocyte/macrophage cell line (4), and zinc pretreatment attenuates TNF-α production during liver injury in mice, thereby protecting against...
Zinc ions influence the cellular levels of cyclic nucleotides. A, Mono Mac1 cells were treated, as indicated, with F SK (10 μM) plus IBMX (4 mM) or ZnSO₄ (25 μM) plus pyrithione (50 μM) for 6 h. Cellular lysates were analyzed as described in Materials and Methods. B, Mono Mac1 cells were treated with ZnSO₄ (25 μM) plus pyrithione (50 μM) and LPS (250 ng/ml) for 30 min. Cells were lysed, and cAMP concentrations were analyzed by cGMP enzyme immunoassay.

The values represent the mean ± SEM from four (A and B) independent experiments, respectively. Statistically significant (p < 0.05) cAMP and cGMP concentration changes are indicated (*).
release was suppressed. However, NO also releases protein-bound zinc by oxidation of zinc-binding cysteine residues (30), and cytokine-induced NO production is sufficient to cause measurable intracellular zinc release (31). Such an increase in available zinc may inhibit cyclic nucleotide degradation via PDE inhibition and act synergistically with the activation of sGC. Because the intracellular concentration of available zinc fluctuates (26), it can be speculated that these fluctuations might influence PDE activity and, thus, cyclic nucleotide signaling. Similar to PDE, zinc inhibits protein tyrosine phosphatases at low micromolar concentrations in cellular lysate (32). An inhibition of these phosphatases by cellular zinc has been shown to participate in the regulation of insulin and insulin-like growth factor-I-modulated tyrosine phosphorylation (32), and it would be interesting to determine whether PDE are affected by cellular zinc in a similar fashion. Plasma contains 12–16 μM zinc (1), and the average concentration of zinc in tissues such as skeletal muscle, kidney, and liver is several hundred micromolar (33). Zinc even reaches millimolar concentrations in zinc-rich tissue such as prostate (34). At these concentrations, PDE should be permanently inactive. Nevertheless, most of that zinc is tightly bound to proteins, and the amount available to inhibit PDE is much lower, but zinc can easily reach concentrations that block TNF-α release in cultured cells and in vivo (4, 6).

Zinc does not inhibit TNF-α and IL-1β secretion, but, rather, affects cytokine mRNA levels. In what way a rise in cGMP influences the signal transduction that leads to the transcription of TNF-α and IL-1β remains to be clarified. The major cGMP target in many cell types is protein kinase G, and cGMP-dependent inhibition of Raf-1 by phosphorylation on serine 43 by protein kinase G has been reported (35). High cGMP concentrations can cross-activate protein kinase A and lead to ERK as well as Raf-1 inhibition (36, 37). Also, destabilization of TNF-α mRNA by cGMP in LPS-activated macrophages was discussed previously (38). The inhibition of TNF gene expression by atrial natriuretic peptide has been found to be a cGMP-mediated effect, involving inhibition of AP-1 and NF-κB activation (12, 39). Which of these mechanisms contribute to zinc inhibition of LPS-induced cytokine production, and the possible involvement of other signaling pathways, are currently under investigation.

Under the experimental conditions used in this study, zinc exerts short-term anti-inflammatory effects via cGMP signals. Down-regulation of PDE-4 expression shows the ability of zinc to act as a modulator of cAMP metabolism as well. PDE-4 is particularly abundant in cells of the immune system, and its inhibitors are investigated as potential anti-inflammatory and immunomodulatory agents to inhibit the synthesis of proinflammatory cytokines and reactive oxygen species. Potential therapeutic targets for PDE-4 inhibition include not only respiratory diseases such as asthma and chronic obstructive pulmonary disease, but also multiple sclerosis, rheumatoid arthritis (RA), diabetes, and septic shock (40, 41). Many of these diseases, such as asthma (42), chronic obstructive pulmonary disease (43), RA (44), and diabetes (45) are associated with reduced serum zinc levels. Elderly individuals, who generally tend toward reduced serum zinc levels, have a significantly increased constitutive production of IL-1, IL-6, and IL-8 as well as an elevated release of these cytokines after stimulation of whole blood samples with LPS compared with young donors (46). Serum levels of TNF-α and IL-1β correlate negatively with the zinc status in RA patients (44). These two
cytokines are also potential candidates for β-cell damage in type 1 diabetes mellitus (45), and zinc supplementation prevents spontaneous and streptozotocin-induced diabetes in NOD mice (47). Therapeutic administration of zinc or, more generally, a correction of a disturbed zinc homeostasis to restore the plasma zinc levels to normal may be ways to modulate cyclic nucleotide levels and thus achieve beneficial results in several inflammatory diseases. The low acute toxicity of zinc indicates that zinc supplementation could be a potential therapy with few side effects. However, the doses, manners, and times of administration of zinc will have to be carefully taken into consideration, because the concentration-dependent pro- or anti-inflammatory effects harboring potentially harmful consequences. This is best demonstrated by the effects of zinc administration during septic shock. Within a narrow concentration range, zinc exerts protective effects in endotoxin-challenged mice (48). Although pretreatment with zinc protects against mortality, simultaneous administration of zinc and LPS increases TNF-α serum levels, resulting in severe proinflammatory action of zinc and enhanced mortality (48–50). Zinc has the potential to be an immune modulatory agent, either through the stimulatory effect of zinc on monocytes or by PDE-mediated suppression of proinflammatory cytokine release in autoimmune disease and septic shock. However, only a thorough understanding of zinc metabolism far beyond our present knowledge, including its uptake and distribution as well as its effects on cellular and molecular levels, will allow us to exploit the full immunomodulatory potential of zinc.

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Disclosures

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References


