SHORT COMMUNICATION

# The influence of metals on the expression of surface antigens on human lymphocytes *in vitro*

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**Abstract**

**OBJECTIVES**: Metals have different effects on the immune functions. Through the experimental *in vitro* model, we studied the changes in the activation and co-stimulatory surface markers in human lymphocytes cultivated with selected metal salts.
**METHODS**: Whole human blood was cultivated with cadmium (Cd) or zinc (Zn) sulfate for 18 hours. The number of lymphocytes positive for activation and co-stimulatory markers was evaluated by flow cytometry.
**RESULTS**: Elevation of the CD69 and CD23 markers as well as higher expression of CD28 was found in cultures of lymphocytes incubated with Cd. In cultures incubated with Zn, minor elevation of the HLA-DR antigen expression was observed in comparison to Cd-treated cell cultures. Decrease of CD3 expression was observed after cultivation with both Cd and Zn salts.
**CONCLUSION**: Cd and Zn exhibit different effects on the expression of human surface activation antigens and co-stimulatory molecules. Cd in non-toxic concentrations stimulated expression of early activation molecules and therefore could change the early phase of immune response. This was not the case for Zn, where the results were similar to untreated cell cultures.

## Introduction

In the field of immunotoxicology, several *in vivo* and *in vitro* methods are available to study the effects of toxic substances on cells of the immune system [7]. Currently, the effects of cadmium (Cd) and zinc (Zn) on the immune system are not entirely known [2, 4, 9]. It has been postulated that Cd might suppress TH1 and stimulate TH2 lymphocytes [5]. In our previous work we used sulfate salts of Cd and Zn ($10^{-2}$ M-$10^{-10}$ M) to study CD69 antigen expression and blast transformation of human lymphocytes [7]. When used in concentrations of $10^{-7}$ M both metals exhibited similar effect. However, if higher metal concentrations were used ($10^{-6}$ M), Cd and Zn had opposite effects on CD69 antigen expression but similar effects on blast transformation. Therefore, in this study we chose the micro-molar concentration of Cd and Zn sulfates for further evaluation of changes of the expression of activation and co-stimulatory surface molecules after exposure of human lymphocytes to Cd and Zn *in vitro*.

## Material and methods

Whole human blood from 18 patients with pollen allergy (allergic rhinitis) and 20 healthy controls was diluted by cultivation medium (RPMI 1640 HEPES, PAA Laboratories, PAA, Prague, Czech Republic) to the final amount of approximately $1x10^6$ leucocytes. The blood was then cultivated in 24-well tissue culture plates with diluted salts of metals ($3\ CdSO_4 \times 8\ H_2O$, $ZnSO_4 \times 7\ H_2O$, in final concentration of $10^{-6}$ M (0.26 μg Cd/ml and 0.3 μg Zn/ml)) and in control culture (only cultivation medium) during 18 hours at 5% $CO_2$ and 37°C. After cultivation, the cells were labeled by monoclonal antibodies (anti-CD3, CD23, CD28, CD69, HLA-DR, Becton Dickinson, Prague, Czech Republic) for 15 minutes. Erythrocytes were lysed twice for 5 minutes (FACS Lysing Solution, Becton Dickinson), and the expression of lymphocyte surface antigens was measured after washing 3 times with Phosphate Buffered Solution. The number of positively stained lymphocytes was evaluated by flow cytometry (FACS Calibur, Becton Dickinson). Antigen expression on gated lymphocytes from untreated blood cultivated with RPMI 1640 medium only was compared with the antigen expression on cells cultivated with Cd and/or Zn salts (mean percentage of positive cells). Statistic evaluation was performed by Student's paired t-test.

## Results

The results are shown in Table 1. The CD3 antigen expression in untreated cultures was detected on the surface of 64% of gated lymphocytes (range of values 47%–81%). In cultures cultivated with Zn, 58% lymphocytes expressed CD3 marker (range of values 25.5%–78%) and in cultures cultivated with Cd 61% (range of values 10%–81.5%). The expression of CD69 activation molecule in non metal treated cells varied widely (range of values 1%–55%). On average, 4% of cells in Zn-treated cultures and 7% of Cd-treated cultures showed CD69 activation marker. Regarding CD23 marker, the results did not differ significantly in control and Zn-treated cultures, but the marker was increased in Cd cultures. The mean values were 2.8% in control cultures, 2.3% in Zn cultures, and 4% in Cd cultures (significant difference from Zn or RPMI treated cultures, p=0.003 or p<0.001).

The expression of the co-stimulatory molecule CD28 was similar in Zn-treated and control cultures and about half of lymphocytes expressed this marker regardless the presence of metal or not. The CD28 expression on lymphocytes cultivated with Cd was significantly higher (64%, range of values 27–80%, p<0.001). Regarding the HLA-DR antigens, we did not find any significant differences attributed to metal (9% of untreated lymphocytes, 9% of lymphocytes cultivated with Zn, and 7% of lymphocytes cultivated with Cd).

## Discussion

This study shows significant elevation of the CD69, CD23 and CD28 lymphocytes in Cd-treated cultures compared to cultures cultivated with Zn or non metal-treated control cultures. The high variability of the CD69 antigen expression in control cultures is surprising. It may be due to the occurrence of spontaneous stimulation of lymphocytes in the absence of any metal added *in vitro*. The reason for this is unknown. We might speculate that other cells present in whole blood cultures, such as dying neutrophiles, can influence lymphocytes activation *in vitro*. Cd and Zn decreased CD3 expression

**Table 1**. Expression of cell surface antigens on lymphocytes in Zn cultures, Cd cultures, and control cultures

| | CD3 (% positive lymphocytes) | | | CD69 (% positive lymphocytes) | | |
|---|---|---|---|---|---|---|
| | Control | Zn | Cd | Control | Zn | Cd |
| Range of values | 47–81 | 25.5–78 | 10–81.5 | 1–55 | 1–12 | 2–18 |
| Mean±SD | 64±9 | 58±16 | 61±22 | 8.5±12 | 4±3 | 7±4* |

| | CD23 (% positive lymphocytes) | | | CD28 (% positive lymphocytes) | | |
|---|---|---|---|---|---|---|
| | Control | Zn | Cd | Control | Zn | Cd |
| Range of values | 0–5 | 0–5.5 | 1–8 | 25–73 | 23–70 | 27–80 |
| Mean±SD | 2.8±1.5 | 2.3±1.5 | 4±2** | 50±14 | 50±15 | 64±14# |

| | HLA–DR (% positive lymphocytes) | | |
|---|---|---|---|
| | Control | Zn | Cd |
| Range of values | 3–18 | 4–12 | 2.5–11 |
| Mean±SD | 9±4 | 9±4 | 7±2 |

* p<0.001 (difference between Cd and Zn cultures)

** p=0.003 (difference between Cd and Zn cultures), p<0.001 (difference between Cd and control cultures)

# p<0.001 (difference between Cd and control cultures, difference between Cd and Zn cultures)

as compared to control cultures. Higher expression of CD28 antigen on T lymphocytes was found in cultures cultivated with Cd, while the same marker was similar in Zn treated and control cultures. In Zn cultures there was minor elevation of the expression of HLA-DR antigen in comparison to the samples with Cd, but the difference was not significant. Jelovcan and co-workers demonstrated that Cd at doses 0.1 μM inhibited IL4/anti-CD40 induced proliferation of human B cells and production of IgE, while expression of CD69 and CD23 was not affected [3]. Marth and collegues described a dose-dependent effect of Cd on the immune system; Cd stimulated the immune system at low concentrations, while at higher concentrations the effects was inhibitory and suppressive [6]. Boscolo described inhibitory effect of Cd in $10^{-4}$ M and $10^{-5}$ M concentrations, but in the concentration $10^{-6}$M no changes were observed [1]. The same publication shows that Zn salts function as a polyclonal stimulator of human lymphocytes at higher concentrations ($10^{-4}$ M, $10^{-5}$ M).

Our findings suggest that Cd salt in micro-molar concentrations can stimulate lymphocytes, but the same was not true for Zn. This might be due to the fact that the concentration used was too low in comparison to the study of Boscolo [1]. It might be interesting to note that in Czech population the normal concentration range of Cd in the whole blood is 0.2–0.8 μg/l (non-smokers) and 1.4–4.5 μg/l (smokers), and blood concentration of Zn is 4000–6000 μg/l whole human blood [10]. Thus, our data were obtained at usual blood concentrations and might thus be clinically relevant.

In conclusion, our study indicates that metals may affect antigen expression at the early stage of activation in various ways. The results with Cd and Zn may be conditioned by the fact that while Zn is an essential element, Cd is a classically toxic metal.

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