

# Effects of Selenium and Cadmium on Changes in the Gene Expression of Immune Cytokines in Chicken Splenic Lymphocytes

Fengping Xu · Shuang Liu · Shu Li

Received: 15 December 2014 / Accepted: 25 January 2015 / Published online: 5 February 2015  
© Springer Science+Business Media New York 2015

**Abstract** Cadmium (Cd) is an environmental pollutant that is considered to be a potent toxin to organisms. Selenium (Se) has been known for its concomitant biological effects and characteristics with Cd. Due to the lack of the research regarding how the duality of Cd/Se affects immune cytokines in poultry, this paper aims to partly tackle this question. Chicken splenic lymphocytes with Cd ( $10^{-6}$  mol/L CdCl<sub>2</sub>), Se ( $10^{-7}$  mol/L Na<sub>2</sub>SeO<sub>3</sub>), Cd + Se ( $10^{-7}$  mol/L Na<sub>2</sub>SeO<sub>3</sub> and  $10^{-6}$  mol/L CdCl<sub>2</sub>), and a control group were incubated for 12, 24, 36, 48, and 60 h, respectively. At each time point, the cells were collected and the messenger RNA (mRNA) expression levels of interleukin (IL)-1 $\beta$ , IL-2, IL-4, IL-10, IL-17, and interferon- $\gamma$  (IFN- $\gamma$ ) were also examined. Compared with the control group and the Se-alone-treated group, the mRNA expression levels of IL-2, IL-4, IL-10, IL-17, and IFN- $\gamma$  decreased significantly in the Cd-alone-treated group. By contrast, the mRNA expression level of IL-1 $\beta$  markedly increased. Levels of IL-2, IL-4, IL-10, IL-17, and IFN- $\gamma$  in Cd + Se-treated groups were significantly higher than those in Cd-alone-treated groups; however, the levels were not as high as the Se-alone-treated groups and the control group. The mRNA expression level of IL-1 $\beta$  in the Cd + Se-treated group was lower than in the Cd-alone-treated group. The relationships with IL-2, IL-4, and IL-10 were found to be closer in the PC 1 matrix and 3D plot of the principal component analysis (PCA) loadings. IL-17 and IFN- $\gamma$  were closer in the matrix of PC 2. However, IL-1 $\beta$  gene expression appeared to be isolated in the matrix of PC 3. In addition, the results of cytokine cluster analysis showed that IL-2, IL-4, IL-10, IL-17, and IFN- $\gamma$  were in the first group and that IL-1 $\beta$  was in the

second group. Therefore, Se partly attenuate immune toxicity induced by Cd in chicken splenic lymphocytes.

**Keywords** Cadmium · Selenium · Cytokines · Chicken splenic lymphocytes

## Introduction

Cd is known for its comprehensive toxicity to mammals, and numerous experiments have demonstrated that Cd causes various forms of damage to mammalian organs, including the lung, kidney, testis, cardiovascular system, liver, ovaries, and skeletal system [1–3]. The influence of Cd on immune functions has also been widely reported. When mice were exposed to Cd in drinking water, the cell-mediated immune responses were significantly suppressed [4]. Mice exposed to Cd (1.8 mg/kg) experienced a 26 % reduction in their mean relative thymus weight 24 h after exposure and a 61 % reduction 72 h after exposure, but the relative spleen weight increased almost 1.6-fold compared with the control group at 72 h [5]. Cd has been shown to induce apoptosis in murine thymocytes, which is accompanied by a loss in cell viability, significant DNA fragmentation, increased ROS, and mitochondrial membrane depolarization [6]. Cd markedly increased the levels of the *Streptococcus pneumoniae* vaccine expression of T-dependent and T-independent serum antibodies in mice. CD4<sup>+</sup>FoxP3<sup>+</sup>CD25<sup>+</sup> (nTreg) cell percentages have been shown to increase in the spleen and thymus in all Cd-exposed offspring. However, CD8<sup>+</sup>CD223<sup>+</sup> T cells decreased sharply in the spleens in all offspring at 7 weeks of age. These findings suggested that even very low levels of Cd exposure during gestation could result in detrimental impacts on the immune system [7]. Experiments to date have demonstrated that cytokines have an important role in immunomodulation; for

F. Xu · S. Liu · S. Li (✉)  
College of Veterinary Medicine, Northeast Agricultural University,  
Harbin 150030, People's Republic of China  
e-mail: lishu@neau.edu.cn

example, secretion of interleukin (IL)-1 $\beta$  is greatly inhibited when human peripheral blood mononuclear cells are exposed to Cd. These findings suggest that low concentrations of Cd (doses from 0.013 to 13.3  $\mu$ M) exert immunomodulatory effects that are dependent on the cell activation pathway and that Cd polarized the immune response toward type 2 in cells stimulated via T cell receptors [8]. Hemdan et al. indicated that Cd exposure of activated T cells led to a suppression of early TH1 cytokine events and stimulation of the TH2 cytokines IL-4 and/or IL-10. This type-2-biased immune response might raise the possibility of Cd-promoted allergic diseases and clarify previous data on the impaired inactivation of viruses and bacteria exposure to Cd [8]. At present, the influence of Cd on poultry has been reported. Lucia et al. reported that Cd triggered oxidative stress and stimulated mitochondrial metabolism in lower levels of Cd, triggering the repression of genes encoding for catalase and acetyl-CoA carboxylase in higher amounts of Cd in birds of the *Anas platyrhynchos* [9]. Cd accumulation has been shown to increase with age in greylag geese [10], and Cd influenced seminiferous tubule diameters in the male lesser scaup (*Aythya affinis*) in the western boreal forest [11]. Li et al. noted that Cd-induced hepatotoxicity in chickens and oxidative stress and apoptosis have been discovered in the chicken liver [12]. Li et al. have also confirmed that testicular toxicity was induced by dietary Cd in cocks [13]. Yang et al. indicated that the number of apoptotic cells in hen ovaries increased in the Cd-alone-treated group, and extensive damage has been observed in the ovaries [14]. In summary, Cd has exhibited various damage to organs or systems of birds, including effects on immune functions.

Numerous studies have shown that Se has an interesting function in its ability to protect from Cd toxicity in mammals. Jihen et al. reported that Se partially alleviated the damage of Cd toxicity in the liver of the rat, and the same effect was noted in the kidney [15]. Concurrent treatment with Se reduced Cd-induced liver histopathological changes, oxidative stress, overexpression of NO, and apoptosis. These results suggested that the toxic effects of Cd on the liver were partly ameliorated by inorganic Se. Se supplementation has also been shown to modify the distribution of Cd in the liver [12]. Li et al. reported that the toxic effect of Cd on the testes was ameliorated by Se [13]. Chen et al. studied the protective effect of Se on Cd-induced change of HSP genes [16]. Zhao et al. studied the protective effect of Se on Cd-induced changes of selenoprotein K, selenoprotein N, selenoprotein S, and selenoprotein T genes [17]. Liu et al. reported that Se ameliorated Cd-induced brain damage in chickens by regulating iNOS-NO system changes. Therefore, Se could serve as a potential therapy for Cd-induced lesions in chickens [18].

Cd causes spleen atrophy, oxidative stress, DNA damage, and apoptosis in chicken splenic lymphocytes, and many studies have shown that Se protects against Cd toxicity in multiple organs. Our previous studies have confirmed that Se

ameliorated Cd-induced apoptosis in chicken splenic lymphocytes [19]. However, the effects of Cd/Se in chicken splenic lymphocytes' immune cytokines messenger RNA (mRNA) expression have not been reported. In this study, we examined the transcription changes of IL-1 $\beta$ , IL-2, IL-4, IL-10, IL-17, and interferon- $\gamma$  (IFN- $\gamma$ ) in chicken splenic lymphocytes with Cd, Se, and Cd + Se in a medium culture solution, and its possible mechanisms were discussed. We also found that the relationships of IL-2, IL-4, and IL-10 were closer in the matrix of PC 1 with a 3D plot of the principal component analysis (PCA) loadings. IL-17 and IFN- $\gamma$  were closer in the matrix of PC 2. However, IL-1 $\beta$  gene expression was isolated in the matrix of PC 3. In addition, the results of the cytokine cluster analysis showed that IL-2, IL-4, IL-10, IL-17, and IFN- $\gamma$  were the first group while IL-1 $\beta$  was in the second one. These results not only helped unveil the immune toxicology of Cd induction but also provided useful clues to understanding that Se abated the toxic effect of Cd.

## Materials and Methods

### Preparation of Chicken Splenic Lymphocytes' Suspension and Treatment

All of the procedures used in this experiment were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University. The spleens were dissected from Isa brown cocks (60 days old) and were collected aseptically and placed in a sterile phosphate-buffered saline (PBS, 0.1 M phosphate buffer with 0.85 % NaCl, pH 7.2). A single cell suspension was prepared by gently pushing the splenic pulp through a sterile stainless steel mesh with a pore size of 100  $\mu$ M. Cells were washed and resuspended in 5 mL of sterile PBS and were then layered over 5 mL of lymphocytes in separation medium (Tian Jin Hao Yang Biological Manufacture Co. Ltd., China). The splenocyte preparations were enriched by centrifugation (2000 $\times$ g) for 15 min at 18  $^{\circ}$ C. Cells were recovered from the interface, resuspended, and washed two times in 8 mL of cell culture medium (RPMI 1640, Gibco, USA). The cells were suspended in complete cell culture medium [RPMI 1640 containing HEPES and 2 mM glutamine, supplemented with 10 % fetal bovine serum (FBS, Gibco, USA) and 1 % antibiotic-antimycotic solution (Sigma, USA)]. The splenic lymphocytes' density was adjusted to  $1.5 \times 10^6$  cells/mL, and the viability of the freshly isolated cells was always above 95 % (trypan blue exclusion test). To monitor various parameters in the present investigation, the control group was incubated for 12, 24, 36, 48, and 60 h without reagents. Cd ( $10^{-6}$  mol/L, Cd-treated group), Se ( $10^{-7}$  mol/L, Se-treated group), and a mixture of Se ( $10^{-7}$  mol/L) and Cd ( $10^{-6}$  mol/L) (Cd + Se-treated group) were added while cells were in a logarithmic growth phase

and were incubated for 12, 24, 36, 48, and 60 h, respectively. The concentrations of Cd and Se used in this study were in accordance with the results of other studies [16, 20].

### Real-Time Polymerase Chain Reaction

Total RNA was isolated from cells using a Trizol reagent according to the manufacturer's instructions (Invitrogen, USA). The RNA concentrations were determined using the GeneQuant 1300.

The reverse transcription reaction (40  $\mu$ L) consisted of the following: 10  $\mu$ g of total RNA, 1  $\mu$ L of M-MLV reverse transcription, 1  $\mu$ L of RNase inhibitor, 4  $\mu$ L of dNTP, 2  $\mu$ L of Oligo dT, 4  $\mu$ L of dithiothreitol, and 8  $\mu$ L of 5 $\times$  buffer. The procedure of the reverse transcription was performed in accordance with the manufacturer's instructions (Invitrogen, USA). The reverse transcription products (cDNA) were then stored at  $-20^{\circ}\text{C}$  for PCR.

To design primers, we used the chicken IL-1 $\beta$ , IL-2, IL-4, IL-10, IL-17, and IFN- $\gamma$  mRNA GenBank sequence with the accession numbers of NM\_204524.1, AY510091, AJ621249, NM0010044142, AY744450, and NM\_205290.1, respectively. Chicken  $\beta$ -actin (GenBank accession number L08165) was used as a housekeeping gene and an internal reference. Primers (Table 1) were designed using the Oligo 6.0 software and were synthesized by Invitrogen Biotechnology Co. Ltd. in Shanghai, China.

Real-time quantitative reverse transcription PCR was used to detect the mRNA expression of IL-1 $\beta$ , IL-2, IL-4, IL-10, IL-17, and IFN- $\gamma$  gene in cells by using SYBR Premix Ex Taq<sup>TM</sup> (Takara, China), and real-time PCR work was performed in an ABI PRISM 7500 real-time PCR system (Applied Biosystems). The program consisted of 1 cycle at  $95^{\circ}\text{C}$  for 30 s, 40 cycles at  $95^{\circ}\text{C}$  for 5 s and at  $60^{\circ}\text{C}$  for 34 s. Dissociation curves were analyzed using Dissociation Curve 1.0 software (Applied Biosystems) for each PCR

reaction to detect and eliminate the possible primer-dimer and nonspecific amplification. The mRNA relative abundance was calculated according to the method of Pfaffl.

### Statistical Analyses

Statistical analysis of all data was performed using SPSS for Windows (version 18.0.0; SPSS Inc., Chicago, IL, USA). When a significant value ( $P < 0.05$ ) was obtained by one-way analysis of variance, further analysis was carried out. All data showed a normal distribution and passed equal variance testing. Differences between the means were assessed using Tukey's honest significant difference test for post hoc multiple comparisons. Data are expressed as the mean  $\pm$  standard deviation.

Correlation coefficient analysis was used to measure the linear correlation between multiple variables. All data were analyzed using the Pearson's correlation coefficient to measure the strength of the linear correlation of the two cytokines. PCA was applied to analyze the relationships among the examined cytokines. The variables were standardized using the means of the z-scores and then calculated using squared Euclidean distance. Finally, hierarchical clustering was performed with the standardized dataset using the Ward's method.

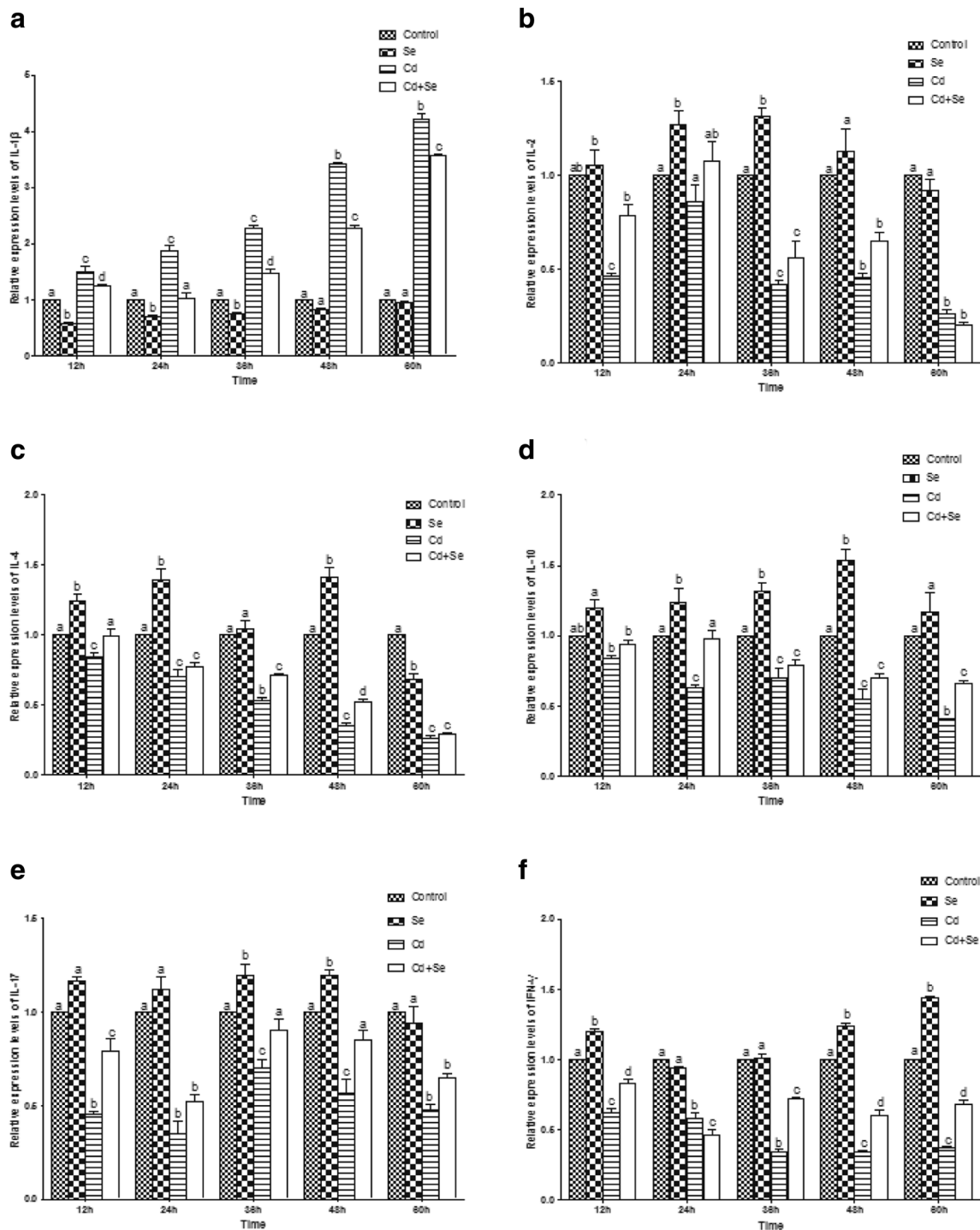
## Results

### The mRNA Expression of IL-1 $\beta$ in Chicken Splenic Lymphocytes

The IL-1 $\beta$  mRNA level in chicken splenic lymphocytes was measured by quantitative RT-PCR (Fig. 1a). Compared with the corresponding control group, a significant increase

**Table 1** Gene special primers used in the real-time quantitative reverse-transcription PCR

Gene	Serial number	Primer sequence	Primer length (bp)	Size of the products (bp)
IL-1 $\beta$	NM_204524.1	Forward 5'-CAGCAGCCTCAGCGAAGAG-3'	19	86
		Reverse 5'-CTGTGGTGTGCTCAGAATCCA-3'	21	
IL-2	AY510091	Forward 5'-GAACCTCAAGAGTCTTACGGGTCTA-3'	25	111
		Reverse 5'-ACAAAGTTGGTCAGTTCATGGAGA-3'	24	
IL-4	AJ621249	Forward 5'-GTGCCACGCTGTGCTTAC-3'	19	82
		Reverse 5'-AGGAAACCTCTCCCTGGATGTC-3'	22	
IL-10	NM0010044142	Forward 5'-CGCTGTCACCGCTTCTCA-3'	19	88
		Reverse 5'-TCCCGTTCTCATCCATCTTCTC-3'	22	
IL-17	AY744450	Forward 5'-CATGTTGTCAGCCAGCATTCT-3'	22	107
		Reverse 5'-CATCTTTTGGGTTAGGCATCC-3'	22	
IFN- $\gamma$	NM_205290.1	Forward 5'-CATCTTTTGGGTTAGGCATCC-3'	22	158
		Reverse 5'-ACTGGATGGCTGGCTTGG-3'	18	
$\beta$ -Actin	L08165	Forward 5'-CCGCTCTATGAAGGCTACGC-3'	20	128
		Reverse 5'-CTCTCGGCTGTGGTGGTGAA-3'	20	



**Fig. 1** The mRNA Levels of IL-1 $\beta$ , IL-2, IL-4, IL-10, IL-17, and IFN- $\gamma$  in chicken splenic lymphocytes. The relative mRNA expression levels from the control group were used as the reference values, and the different

letters indicate that there were significant differences ( $P < 0.05$ ) between any two groups. Each value represented the mean  $\pm$  SD of five individuals

( $P < 0.05$ ) in the expression of the IL-1 $\beta$  mRNA level was observed in the Cd-alone-treated groups at 12, 24, 36, 48, and 60 h. The increase in IL-1 $\beta$  mRNA expression was in a time-dependent manner. Se supplementation was able to partly reverse this status but did not return to the levels of the control group.

### The mRNA Expression of IL-2 in Chicken Splenic Lymphocytes

As shown in Fig. 1b, the mRNA expression of IL-2 significantly decreased ( $P < 0.05$ ) in the Cd-treated group compared with the corresponding control group and the Se-treated



group. The Se-treated group increased but was not significantly different at 12 h. The relative mRNA expression of IL-2 in the Cd + Se-treated group was in between the Se-alone-treated group and the Cd-alone-treated group at 12, 24, 36, and 48 h, but there were no significant differences ( $P>0.05$ ) between the Cd + Se-treated group and Cd-alone-treated groups at 60 h after exposure.

#### The mRNA Expression of IL-4 in Chicken Splenic Lymphocytes

As shown in Fig. 1c, the mRNA levels of IL-4 significantly decreased ( $P<0.05$ ) in the chicken splenic lymphocytes of the Cd-alone-treated group compared with the corresponding control group at 12, 24, 36, 48, and 60 h after exposure. The increase in IL-4 mRNA expression was in a time-dependent manner. In the Cd + Se-treated group, the IL-4 mRNA expression level improved significantly compared with the Cd-alone-treated group but was not restored to the levels of the control group and the Se-alone-treated group.

#### The mRNA Expression of IL-10 in Chicken Splenic Lymphocytes

As shown in Fig. 1d, compared with the corresponding control group, a significant decrease ( $P<0.05$ ) in the mRNA level of IL-10 mRNA level was observed in all Cd-alone-treated groups. The IL-10 mRNA expression increased in a time-dependent manner. Se supplementation was able to partly reverse the status, but the IL-10 mRNA expression level did not return to the levels of the control group and the Se-alone-treated group.

#### The mRNA Expression of IL-17 in Chicken Splenic Lymphocytes

The IL-17 mRNA level in the chicken splenic lymphocytes was measured by quantitative RT-PCR (Fig. 1e). The level in the Se-alone-treated group increased but was not significantly different at 12, 24, and 60 h and was reduced significantly in the Cd-alone-treated group compared with the control group at each time point. The relative mRNA level of IL-17 of the Cd + Se-treated group was between in the Se-alone-treated group and the Cd-alone-treated group. IL-17 mRNA expression of the Cd-treated group increased gradually and then decreased in all groups.

#### The mRNA Expression of IFN- $\gamma$ in Chicken Splenic Lymphocytes

The IFN- $\gamma$  mRNA level in the chicken splenic lymphocytes was measured by quantitative RT-PCR (Fig. 1f). The IFN- $\gamma$  mRNA level showed no statistically significant difference

( $P>0.05$ ) between the control group and the Se-treated group at 24 and 36 h. Compared with the corresponding control group, a significant decrease ( $P<0.05$ ) of the IFN- $\gamma$  mRNA expression level was observed in all of the Cd-alone-treated groups. The status was reversed partly by Se but did not return to the levels of the control group.

#### Chemometrics

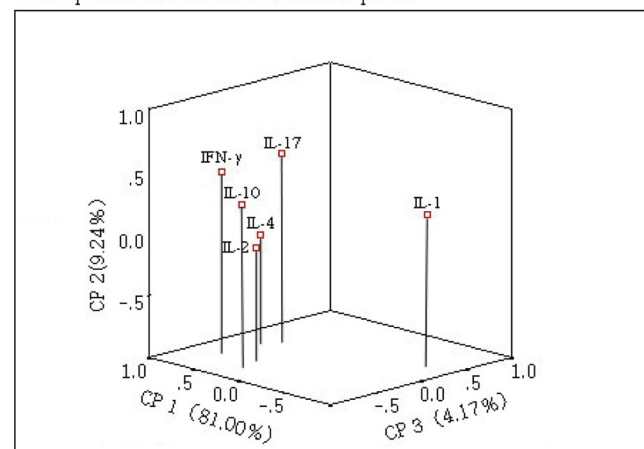
The six different cytokine expressions were analyzed from 20 samples of five time points and four treatment groups of chicken spleen lymphocytes. A 3D plot of the PCA loadings is presented in Fig. 2 which clearly indicates that IL-2, IL-4, and IL-10 were closer to the space distance between each other in the matrix of PC 1, meaning that they had closer relationships. IL-17 and IFN- $\gamma$  were closer to the space distance between each other in the matrix of PC 2, meaning that their relationships were closer. However, IL-1 $\beta$  gene expression was isolated in the matrix of PC 3 and was far away from these interleukins.

The results of the cytokine cluster analysis are shown in Fig. 3. The cluster had the following two groups: the first group contained IL-2, IL-4, IL-10, IL-17, and IFN- $\gamma$ , and the second one included only IL-1 $\beta$ .

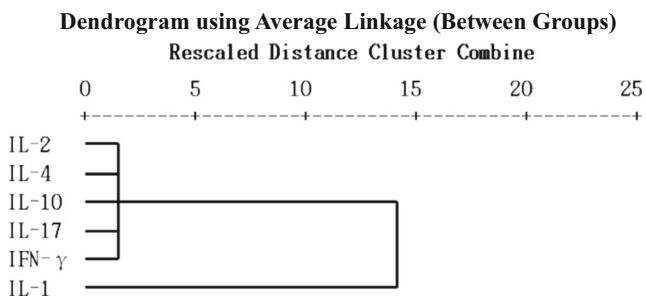
#### Discussion

Many heavy metals might cause functional alterations to the immune system via humoral and cellular immune responses. After exposure to excessive manganese, IL-1 $\beta$  and IL-2 mRNA levels have been shown to decrease in immune organs. The immune system of cocks were shown to be injured, and the immune functions were also suppressed due to manganese [21]. Van Ooik et al. demonstrated that Ni and Cu directly

Component Plot in Rotated Space



**Fig. 2** A 3D plot of the PCA of cytokines measured in chicken splenic lymphocytes after exposure to Cd ( $n=20$ )



**Fig. 3** Dendrogram resulting from the Ward's method of hierarchical cluster analysis for the six variables and the 20 samples. Similarities have been calculated from the Euclidean squared distance

inhibit immune function [22]. Lafuente et al. reported that B and T lymphocytes decreased in peripheral blood with 5 and 10 ppm of CdCl<sub>2</sub> in drinking water [23]. Dong et al. reported that Cd was able to cause apoptosis in mouse thymocytes in a time- and dose-dependent manner and that Cd treatment also altered thymocyte surface marker expression, leading to evident phenotypic changes [24]. The susceptibility to Cd-induced toxicity was influenced by a number of factors. Se is an important nutritional trace element. It is primarily incorporated into selenoproteins as the amino acid selenocysteine to perform its biological functions [25, 26]. Se may not only play roles in some tissues, such as the liver, pancreas, muscles, neutrophils, and thyroids [27–32], but may also have important roles in immune responses [33]. Moreover, Se has been shown to biologically interact with Cd. Many studies have indicated that Se could protect against Cd toxicity. Because cytokines could provide important information of immune function, we cultivated chicken splenic lymphocytes in vitro and tested the cytokine expression (including IL-1β, IL-2, IL-4, IL-10, IL-17, and IFN-γ) to reflect the immune function. We found that the mRNA expression of IL-2, IL-4, IL-10, IL-17, and IFN-γ decreased at 12–60 h after exposure in the Cd-alone-treated group, while the mRNA expression of IL-1β increased. In the Cd + Se-treated group, the mRNA expressions of IL-2, IL-4, IL-10, IL-17, and IFN-γ were higher than the Cd-alone-treated group; however, the levels were not as high as the Se-alone-treated group and the control group. These results indicated the protective effect of Se against the toxicity of Cd.

IL-1 is secreted by activated T lymphocytes. This cytokine is an important mediator of the inflammatory response and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis [33]. IL-1β (pro-inflammatory cytokines) is a member of the IL-1 cytokine family. The upregulation of IL-1β induced by simvastatin led to immunosuppression in human T cells in vitro [34]. Marth et al. reported that the highest accumulation of mRNA was found in peripheral blood mononuclear cells (PBMCs) in adults exposed to 5 μmol/L of CdCl<sub>2</sub>. A 5–6-fold increase in IL-1β mRNA levels was observed compared with

the control group, and the inhibition of IgE synthesis reflected the suppression of immune function [35]. Our results showed that Cd could lead to an increase in the levels of IL-1β increased, indicating certain immune damage with Cd. The mRNA expression levels of IL-1β in the Cd + Se-treated group were lower than that of the Cd-alone-treated group. Similar to these result, Seda et al. reported that selenium at 0.625 mg/kg/day decreased IL-1β levels during the treatment of ischemia-reperfusion-induced brain injury in rats [36]. It is possible that Se attenuated Cd-induced immune toxicity through reducing the levels of IL-1β.

IL-2 is widely recognized as a T cell growth factor and plays an important role in immunoregulation. In addition to promoting T cell proliferation, IL-2 increases cytokine production and enhances proliferation of B cells and natural killer cells, modifying the functional properties of fibroblasts and possibly macrophages, thereby contributing to immune responses. Decreased IL-2 levels are associated with a general decline in immune function and immune regulation [37]. IL-2 mRNA levels decreased when Hyline cocks were fed with Mn-supplemented diet [21]. These findings suggested that decreases of IL-2 could result in detrimental effects on the immune system. Our results showed that the mRNA expression levels of IL-2 in the Cd + Se-treated group were higher than the Cd-alone-treated group. The results agreed with Montgomery et al., who suggested that Se might enhance the immunity of young foals through increasing the expression of IL-2; they also found that IL-2 was essential for lymphocyte proliferation [38]. Se attenuated Cd-induced immune toxicity through reducing the mRNA levels of IL-2. IL-4 and IL-10, which are the key cytokines secreted by Th2 lymphocytes and are considered to be a pleiotropic anti-inflammatory cytokine produced by activated CD4-T cells, mast cells, eosinophils and basophils. These cells could affect T and B lymphocytes, natural killer cells, mast cells, synoviocytes, and endothelial cells. They could activate and support the growth of B cells and trigger the production of IgG and IgE [39]. Our results showed that the levels of mRNA expression of IL-4 and IL-10 decreased significantly ( $P < 0.05$ ) in chicken splenic lymphocytes of the Cd-alone-treated group compared with the corresponding control groups at 12, 24, 36, 48, and 60 h. However, Hemdan et al. have confirmed that the expression of IL-4 and IL-10 were enhanced at lower doses of Cd from 13.3 to 133 nM in human peripheral blood mononuclear cells [8]. Applying Cd under the same experimental conditions led to a significant increase in the levels of IL-4 and IL-10 [40]. These studies were not consistent with our results and may at least be in part due to the different experimental conditions used, such as different application regimes or varying cell models. IL-17 is a pro-inflammatory cytokine that plays an essential role in host defenses against microbial infections and is implicated in various inflammatory conditions, such as autoimmune diseases, metabolic disorders, and cancer [41].

Jameel et al. confirmed that the levels of IL-17 production and immune function have a positive relationship. IL-17 decreased when immune function was reduced in human T cell responses *in vitro* [34]. In our experiment, the IL-17 mRNA expression of the Cd + Se-treated group was higher than in the Cd-treated group; however, the expression was not as high as the Se-alone-treated groups and the control group. IFN- $\gamma$  regulates both innate and acquired immunities by participating in host defenses or pathogenesis, such as sepsis syndrome and autoimmune diseases. Expression of the IFN- $\gamma$  gene decreased in all of the samples tested at doses from 133 nM to 13.3  $\mu$ M [8]. Montgomery et al. suggested that Se might enhance the immunity of young foals through increased expression of IFN- $\gamma$  and that Se was essential for cellular immune response [38]. Our results agreed with previous data as IFN- $\gamma$  levels were higher in the Cd + Se-treated group compared with the Cd-alone-treated group.

In summary, our results indicated that the mRNA expression levels of IL-2, IL-4, IL-10, IL-17, and IFN- $\gamma$  significantly decreased and IL-1 $\beta$  significantly increased in the presence of Cd. On the other hand, Se attenuated Cd-induced immune toxicity. We also found that IL-2, IL-4, IL-10, IL-17, IFN- $\gamma$ , and IL-1 $\beta$  had a negative relationship. IL-2, IL-4, IL-10, IL-17, and IFN- $\gamma$  belonged to the first group, and IL-1 $\beta$  belonged to the second group. Therefore, these results indicated that Cd suppressed immune function and Se partly attenuate immune toxicity induced by Cd in chicken splenic lymphocytes. The information presented in this study is helpful to understanding the mechanism of Cd-induced immunotoxicity and the protective role of Se.

**Acknowledgments** This study was supported by the National Natural Science Foundation of China (Grant No. 31472161).

**Conflict of Interest** The authors declare no conflicts of interest.

## References

1. Angenard G, Muczynski V, Coffigny H et al (2010) Cadmium increases human fetal germ cell apoptosis. *Environ Health Perspect* 118(3):331–337
2. Brzóska MM, Moniuszko-Jakoniuk J (2005) Disorders in bone metabolism of female rats chronically exposed to cadmium. *Toxicol Appl Pharmacol* 202(1):68–83
3. Modi HR, Patil N, Katyare SS (2008) Effect of treatment with cadmium on kinetic properties of Na<sup>+</sup>, K<sup>+</sup>-ATPase and glucose-6-phosphatase activity in rat liver microsomes a correlative study on influence of lipid/phospholipid make-up. *Toxicology* 254(1–2):29–41
4. Dan G, Lall SB, Rao DN (2000) Humoral and cell mediated immune response to cadmium in mice. *Drug Chem Toxicol* 23(2):349–360
5. Pathak N, Khandelwal S (2007) Role of oxidative stress and apoptosis in cadmium induced thymic atrophy and splenomegaly in mice. *Toxicol Lett* 169(2):95–108
6. Pathak N, Khandelwal S (2006) Influence of cadmium on murine thymocytes: potentiation of apoptosis and oxidative stress. *Toxicol Lett* 165(2):121–132
7. Hanson ML, Holásková I, Elliott M et al (2012) Prenatal cadmium exposure alters postnatal immune cell development and function. *Toxicol Appl Pharmacol* 261(2):196–203
8. Hemdan NY, Emmrich F, Sack U et al (2006) The *in vitro* immune modulation by cadmium depends on the way of cell activation. *Toxicology* 222(1–2):37–45
9. Lucia M, Andre JM, Gonzalez P et al (2010) Effects of dietary cadmium contamination on bird *Anas platyrhynchos*—comparison with species *Cairina moschata*. *Ecotoxicol Environ Saf* 73(8):2010–2016
10. Lucia M, André JM, Gontier K et al (2010) Trace element concentrations (mercury, cadmium, copper, zinc, lead, aluminium, nickel, arsenic, and selenium) in some aquatic birds of the southwest Atlantic coast of France. *Arch Environ Contam Toxicol* 58(3):844–853
11. Pollock B, Machin KL (2008) Effects of cadmium, mercury, and selenium on reproductive indices in male lesser scaup (*Aythya affinis*) in the western Boreal forest. *Arch Environ Contam Toxicol* 54(4):730–739
12. Li JL, Jiang CY, Li S et al (2013) Cadmium induced hepatotoxicity in chickens (*Gallus domesticus*) and ameliorative effect by selenium. *Ecotoxicol Environ Saf* 96(103–109)
13. Li JL, Gao R, Li S et al (2010) Testicular toxicity induced by dietary cadmium in cocks and ameliorative effect by selenium. *Biomaterials* 23(4):695–705
14. Yang SH, Zhang ZW, He JB et al (2012) Ovarian toxicity induced by dietary cadmium in hen. *Biol Trace Elem Res* 148(1):53–60
15. Jihen el H, Imed M, Fatima H et al (2008) Protective effects of selenium (Se) and zinc (Zn) on cadmium (Cd) toxicity in the liver and kidney of the rat: histology and Cd accumulation. *Food Chem Toxicol* 46(11):3522–3527
16. Chen X, Zhu YH, Cheng XY et al (2012) The protection of selenium against cadmium-induced cytotoxicity via the heat shock protein pathway in chicken splenic lymphocytes. *Molecules* 17(12):14565–14572
17. Zhao W, Liu W, Chen X et al (2014) Four endoplasmic reticulum resident selenoproteins may be related to the protection of selenium against cadmium toxicity in chicken lymphocytes. *Biol Trace Elem Res* 161(3):328–333
18. Liu LL, Li CM, Zhang ZW et al (2014) Protective effects of selenium on cadmium-induced brain damage in chickens. *Biol Trace Elem Res* 158(2):176–185
19. Liu S, Xu FP, Yang ZJ et al (2014) Cadmium-induced injury and the ameliorative effects of selenium on chicken splenic lymphocytes: mechanisms of oxidative stress and apoptosis. *Biol Trace Elem Res* 160(3):340–351
20. Ruan H, Zhang Z, Wu Q et al (2012) Selenium regulates gene expression of selenoprotein W in chicken skeletal muscle system. *Biol Trace Elem Res* 145(1):59–65
21. Liu X, Li Z, Han C et al (2012) Effects of dietary manganese on Cu, Fe, Zn, Ca, Se, IL-1 $\beta$ , and IL-2 changes of immune organs in cocks. *Biol Trace Elem Res* 148(3):336–344
22. Van Ooik T, Pausio S, Rantala MJ (2008) Direct effects of heavy metal pollution on the immune function of a geometrid moth, *Epirrita autumnata*. *Chemosphere* 71(10):1840–1844
23. Lafuente A, González-Carracedol A, Esquifino AI (2004) Differential effects of cadmium on blood lymphocyte subsets. *Biomaterials* 17(4):451–456
24. Dong S, Shen HM, Ong CN (2001) Cadmium-induced apoptosis and phenotypic changes in mouse thymocytes. *Mol Cell Biochem* 222(1–2):11–20
25. Yao HD, Liu W, Zhao W (2014) Different responses of selenoproteins to the altered expression of selenoprotein W in chicken myoblasts. *RSC Advances* 4(64032–64042)

26. Yao HD, Wu Q, Zhang ZW et al (2013) Selenoprotein W serves as an antioxidant in chicken myoblasts. *Biochim Biophys Acta* 1830(4): 3112–3120
27. Naziroğlu M, Karaoğlu A, Aksoy AO (2004) Selenium and high dose vitamin E administration protects cisplatin-induced oxidative damage to renal, liver and lens tissues in rats. *Toxicology* 195(2–3):221–230
28. Zhao X, Yao H, Fan R et al (2014) Selenium deficiency influences nitric oxide and selenoproteins in pancreas of chickens. *Biol Trace Elem Res* 161(3):341–349
29. Yao HD, Wu Q, Zhang ZW et al (2013) Gene expression of endoplasmic reticulum resident selenoproteins correlates with apoptosis in various muscles of se-deficient chicks. *J Nutr* 143(5):613–619
30. Yao HD, Zhao W, Zhao X et al (2014) Selenium deficiency mainly influences the gene expressions of antioxidative selenoproteins in chicken muscles. *Biol Trace Elem Res* 161(3): 318–327
31. Chen X, Yao H, Yao L et al (2014) Selenium deficiency influences the gene expressions of heat shock proteins and nitric oxide levels in neutrophils of broilers. *Biol Trace Elem Res* 161(3):334–340
32. Lin SL, Wang CW, Tan SR et al (2014) Selenium deficiency inhibits the conversion of thyroidal thyroxine (T4) to triiodothyronine (T3) in chicken thyroids. *Biol Trace Elem Res* 161(3):263–271
33. Hoffmann PR, Berry MJ (2008) The influence of selenium on immune responses. *Mol Nutr Food Res* 52(11):1273–1280
34. Jameel A, Ooi KG, Jeffs NR et al (2013) Statin modulation of human T-cell proliferation, IL-1 $\beta$  and IL-17 production, and IFN- $\gamma$  T cell expression: synergy with conventional immunosuppressive agents. *Int J Inflam* 2013(434586)
35. Marth E, Jelovcan S, Kleinhappl B et al (2001) The effect of heavy metals on the immune system at low concentrations. *Int J Occup Med Environ Health* 14(4):375–386
36. Ozbal S, Erbil G, Kocdor H et al (2008) The effects of selenium against cerebral ischemia-reperfusion injury in rats. *Neurosci Lett* 438(3):265–269
37. Malek TR, Yu A, Zhu L et al (2008) IL-2 family of cytokines in T regulatory cell development and homeostasis. *J Clin Immunol* 28(6): 635–639
38. Montgomery JB, Wichtel JJ, Wichtel MG et al (2012) Effects of selenium source on measures of selenium status and immune function in horses. *Can J Vet Res* 76(4):281–291
39. Uceyler N, Valenza R, Stock M et al (2006) Reduced levels of antiinflammatory cytokines in patients with chronic widespread pain. *Arthritis Rheum* 54(8):2656–2664
40. Krocova Z, Macela A, Kroca M et al (2000) The immunomodulatory effect(s) of lead and cadmium on the cells of immune system in vitro. *Toxicol In Vitro* 14(1):33–40
41. Ouyang W, Kolls JK, Zheng Y (2008) The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity* 28(4): 454–467