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Effect of cadmium on immune responses and enzyme activities of BALB/c mice

1. Cellular immune responses

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카드뮴이 BALB/c 마우스의 면역반응 및 효소활성에 미치는 영향

1. 세포성 면역반응

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초록 : 카드뮴이 BALC/c 마우스의 세포성면역능에 미치는 영향을 평가하고자 여러 농도의 카드뮴이 첨가된 음료를 장기간 동안 자유급식하고, 비장내 T helper 및 T suppressor cell의 분포도, 복강대식세포의 탐식능 및 시험관내에서 Con-A 및 LPS에 대한 비장세포의 증식능을 조사하여 다음과 같은 결과를 얻었다.

1. 각군의 6주간 체중 증가율은 대조군이 27.0%이었으며, 25, 50, 100 및 200ppm $CdCl_2$ 투여군의 체중증가율은 각각 28.54%, 28.31%, 20.49% 및 18.04%로 나타났다.

2. 체중당 비장무게(mg/g)는 대조군이 4.34±0.23이었으며, 25, 50, 100 및 200ppm $CdCl_2$ 투여군은 각각 4.20±0.54, 4.80±0.87, 4.25±0.32 및 4.40±0.32이었다. 또한 총비장세포수($\times 10^7$)는 대조군(24.29±5.98)에 비해 25, 50, 100 및 200ppm $CdCl_2$ 투여군에서 각각 14.1%, 35.7%, 16.6% 및 22.0% 증가하였다.

3. 총 CD_4^+ 세포수($\times 10^7$)는 대조군이 9.15±2.24, 25, 50, 100및 200ppm $CdCl_2$ 투여군은 각각 10.40±2.04, 12.04±3.08, 10.20±3.16 및 10.80±1.48 이었으며, 총 CD_8^+ 세포수($\times 10^7$)는 대조군이 2.32±0.56, 각 실험군의 총세포수는 2.54±0.27, 3.12±0.80, 2.25±0.70 및 2.24±0.28 이었다. 한편, CD_4^+/CD_8^+ 비율은 50ppm 투여군(3.88±0.01)을 제외하고 모든 실험군에서 대조군(3.97±0.02)에 비해 유의하게 증가하였다($p<0.001$).

4. 카드뮴 처리군의 SRBC에 대한 복강대식세포의 탐식능은 25 및 50ppm 투여군에서는 대조군에 비하여 유의하게 높았으나($p<0.05$ 및 $p<0.01$), 100 및 200ppm 투여군에서는 대조군과 유사하였다.

5. 시험관에서의 Con-A 및 LPS에 대한 비장림프구 증식반응은 LPS의 경우 카드뮴 농도에 비례하여 억제되었으나 Con-A에 대한 증식반응은 저농도(10^{-7}-10^{-6}M)에서 증가된 양상을 보였다.

6. $CdCl_2$에 대한 비장세포의 세독독성은 10^{-4}M에서 특히 높았다.

이상의 결과는 카드뮴이 세포성면역반응에서 중요한 역할을 하는 T세포 아군의 분포도를 변화시키므로써 면역반응에 영향을 줄 수 있음을 시사한다.

Key words : cadmium, cytotoxicity, mitogens, Th/Ts ratio, phagocytosis, mouse

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Introduction

Cadmium(Cd) is a potential hazard in industrialized and urbanized societies. It is increasing use in industry and imminent danger of contamination and pollution from these industries exits[1-6]. At present, the major hazards of Cd would apper to be those resulting from exposure to dust or fumes contanining high concentration of the metal, or from foods contaminated by Cd which was common[3]. A condition known as Itai-Itai disease, found in Japanese living near the Jintsu River, was traced to Cd toxicity[2,3]. Cd is also a contaminant of superphosphate fertilizers and is known to be absorbed by plant roots and transported to other plant parts[1,3].

Acute or chronic exposure to Cd induces damage to the liver, kidney and to the central nervous system, testicular atrophy and teratogenic effects. And also it produces anemia, anosmia, hypersensitivity, infertility, osteomalacia, emphysema, pulmonary cancer and autoimmunity etc[1,4,7,9,11,21,33].

There is also accumulating evidence for immune alteration in animals exposed to Cd. Cd exposure has been reported variously to stimulate[10,12-14] to suppress[15-19], and have no effect on the immune responses[20]. These diverse effects might be related to the dose, route, time of administration with respect to antigen stimulation, length of exposure to Cd and strains of Cd intoxicated animals[20,21]. Meanwhile, the proliferative responses of splenocytes to various mitogens were decreased[24,25] or increased[10,14,22,23] by heavy metals administration. As described above, there are a lot of controversy about the effects of Cd on immune responses. And also, the degrees of Cd accumulation in organs which were related with immune responses such as blood and spleen were different from each other[26].

On the other hand, it was reported that blood T-lymphocytes was reduced in Cd-fed mice. This result might be associated with the increase of the number of splenic T-lymphocytes, namely Cd exposure modified distribution of lymphocyte subpopulations(T/B ratios)[27]. Thomas et al[24] reported that Cd administration activated T-suppressor cells by augmenting macrophage activity, subsequently it suppressed the immune responses of lymphocytes. These reports suggest that Cd exposure is able to affect the distribution of T heper and T suppressor cells in blood and lymphoid organs.

However, there were few reports that distribution of splenic T-cell subpopulations was modifid by Cd exposure. To clarify the effects of Cd in the cellular immune responses, Cd was administered to BALB/c mice for 6 weeks via drinking water *ad libitum* since the most common route of chronic exposure to Cd in animals is oral route. In addition, to correlate changes in host resistance with the state of immunity at the time of antigen challenge several measures of immune functions such as phagocytosis of peritoneal macrophages, blastogenesis and cytotoxicity of splenocytes were also made.

In this manuscript, the effects of Cd administered *ad libitum* on the cellular immune responses in BALB/c mice were investigated.

Materials and Methods

Animals : Male BALB/c mice, 6 to 8 weeks of age and weighing 17 to 25g, were obtained from the Korea Research Institute of Chemical Technology (Taejeon). Animals were housed five to six per cage, maintained at ambient temperatures of 20 to 23℃ and fed commercial rodent chow pellet(Sam-yang Co) *ad libitum*.

Cadmium treatment : Cadmium chloride($CdCl_2$) was provided by Sigma(C-3141). For *in vivo* exposure to Cd, mice were received distilled water alone or water supplemented with 25, 50, 100 or 200ppm $CdCl_2$ *ad libitum* for 6 weeks. For *in vitro* treatment of Cd, $CdCl_2$ was added to the mitogen-stimulating cultures or the culture for cytotoxicity test at the initation of culture to give final concentrations of 10^{-7} to 10^{-4}M.

Body and relative organ weight determinations : Animals were weighed weekly for the duration of the study. One day following Cd exposure, blood was drained by puncture of orbital venous plexus, and spleens were removed, trimmed excess connective tissues and weighed. Results were represented as organ

per body weight ratio(mg/g) for the spleen.

Spleen cell suspension and splenic cellularity : Spleens were removed from control and Cd-intoxicated mice. Single cell suspensions of the spleen were prepared after lysing RBCs in distilled water. The culture medium was RPMI-1640 supplemented with penicillin(100IU/ml), streptomycin(100μg/ml), 2mM L-glutamine, 25mM Hepes buffer, and 5% fetal calf serum(FCS, Gibco). Splenic cellularity was determined with hemocytometer on appropriate dilution of cell suspension after teasing. Viability was estimated by trypan blue dye exclusion test[37].

Cytotoxicity test[37]: Various concentrations of $CdCl_2$(10^{-7}-10^{-4}M) were added to 0.1ml culture of splenocytes(2×10^6cells/ml) during various times(2, 4, 6, 8 and 24 hrs) at 37℃ in 96 well culture plate.

$$\text{Cytotoxic index(\%)} = \frac{\text{\% died with } CdCl_2 - \text{\% died without } CdCl_2}{100 - \text{\% died without } CdCl_2} \times 100$$

Assay for mitogen responsiveness[25] : Spleen cells (2×10^5 cells/well) were incubated in 96 well flat-bottomed tissue culture plates(Falcon No 3072) in 0.1ml culture media. A mitogen and/or Cd solution was added at the initiation of culture. Mitogens were added to the cultures to give final concentrations of 4μg/ml for concanavalin-A(Con-A, Sigma) and 16μg/ml for lipopolysaccharide(LPS, Sigma). These concentrations of mitogens produced optimum stimulation of the incubated lymphocytes(Fig 2, 3). The cultures were incubated at 37℃ under 5% CO_2-air for 48 hrs. Lymphocyte transformation was estimated by measuring DNA synthesis as follows: Cells were labeled with 1.0μCi of [^{3}H] thymidine(25Ci/mmol, Amersham, England) per well for 8 hrs before harvesting cells. The cultures were harvested onto glass fiber filters with an automated cell harvester. The radioactivity on the filter disks was counted with a liquid scintillation spectrometry. Proliferative responses were shown as counts per minute(CPM) of [^{3}H] thymidine incorporation per culture.

Phagocytosis assay[38] : One day following Cd treatment, 0.5ml of 1% SRBC suspension in PBS was injected into the peritoneal cavity of the mice. Thirty minutes later, 3ml of PBS was given i.p. The peritoneum was gently massaged and cells were obtained by aspiration. After being incubated at 37℃ for 15min, the cells on the slide were stained with Wright-Giemsa. The percentage of phagocytosis(the percentage of macrophage ingesting SRBC) was calculated.

$$\text{\% of phagocytosis} = \frac{\text{SRBC ingesting macrophages}}{\text{Total macrophages counted}} \times 100$$

Determination of T-cell subpopulations[37] : Spleen cell supensions were prepared and stained with phycoerythrin(PE) conjugated anti-mouse L_3T_4 for T-helper/inducer(CD_4^+) cells and FITC conjugated anti-mouse Lyt-2 for T-suppressor/cytotoxic(CD_8^+) cells (Becton Dickinson). Briefly, 6μl of each monoclonal antibody(Mab) and 44μl of RPMI-1640 were added in tube and mixed. To these mixture, 50μl of cell suspension(2×10^7 cells/ml) was added and reacted in dark area for 40min. Then, washed two times with PBS and fixed with 1% formaldehyde in PBS. Finally, T-cell subpopulations were measured by flow cytometry(FACScan, Becton Dickinson).

Statistical analysis : The analysis of variance (ANOVA) or the Student's t-test was employed.

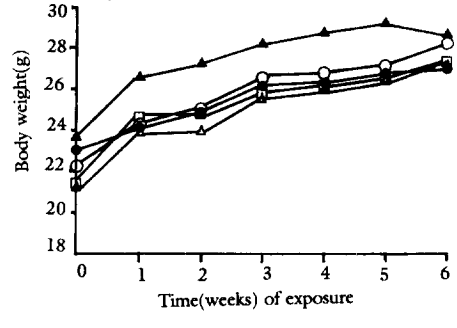

Fig 1. Body weight changes of BALB/c mice orally administered various concentrations of $CdCl_2$ for 6 weeks.
Mice fed distilled water(□-□) or various concentrations of $CdCl_2$, 25ppm(△-△), 50ppm(○-○), 100ppm(▲-▲) and 200ppm(●-●), respectively. Body weight was measured weekly during $CdCl_2$ exposure. Each point represents mean from 6 mice.

Table 1. Effect of $CdCl_2$ on water consumption in BALB/c mice during 6 weeks[a]

$CdCl_2$ in Drinking water(ppm)	Water consumption[b] (ml/mouse/day)	Percentage of Control
0	4.66	100
25	4.73	101.50
50	4.53	97.21
100	4.55	97.64
200	3.46	74.25

[a] : $CdCl_2$ administration in drinking water.

[b] : Mean water consumption from 6 mice in a cage.

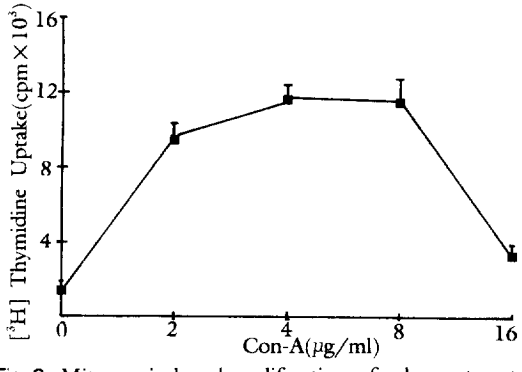

Fig 2. Mitogen-induced proliferation of splenocytes at different concentrations of Con-A.
Splenocytes(2×10^5cells/well) were cultured in culture media containing various concentrations of Con-A. Cells were harvested at 48hrs and assayed [^{3}H] thymidine uptake. Each point represents the mean±SD of triplicate replication from a pool of 3 spleens.

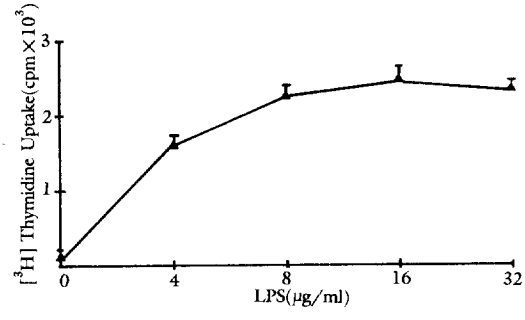

Fig 3. Mitogen-induced proliferation of splenocytes at different concentrations of LPS.
Splenocytes(2×10^5cells/well) were cultured in culture media containing various concentrations of LPS. Cells were harvested at 48 hrs and assayed [^{3}H] thymidine uptake. Each point represents the mean±SD of triplicate replication from a pool of 3 spleens.

Results

Water consumption and changes in body weight : The mice fed 25, 50 and 100ppm Cd drank as much as control, but the mice fed 200ppm Cd drank significantly less water after Cd exposure than did control($p<0.05$).

Loss of body weight is a common sign of toxicity at toxic dose in animals. Changes in body weight of mice of all groups investigated during Cd exposure(6 weeks) were summarized in Table 2. Growth rates of body weight during 6 weeks were as this; control group 27.0%, Cd administered groups(25, 50, 100 and 200ppm) 28.54%, 28.31%, 20.49% and 18.04%, respectively. All groups of mice were in good health with no clinical signs.

Spleen index, cellularity and proportion of CD_4^+ or CD_8^+ cells: Mild splenomegaly was observed in 50 and 200ppm Cd administered groups, although other experimental groups were not shown increase of spleen indices as compared with control(Table 3).

But total number of splenocytes tended to increase

Table 2. Body weight changes of BALB/c mice orally administered various concentration of $CdCl_2$

Weeks	Body weight(g)				
	$CdCl_2$ conc(ppm)				
	0	25	50	100	200
0	21.48±3.35[a]	21.20±2.81	22.08±3.19	23.77±0.97	23.0±1.22
1	24.42±2.81	23.98±1.91	24.24±2.75	26.53±1.23	24.09±1.84
2	24.44±2.72	23.98±2.04	24.74±3.02	27.08±1.29	24.53±2.03
3	25.89±2.57	25.68±1.42	26.28±3.05	28.35±1.57	26.06±2.38
4	26.03±2.42	25.96±1.77	26.66±3.15	28.61±1.71	26.30±2.33
5	26.29±2.31	26.22±2.02	27.03±2.99	29.08±2.00	26.78±2.48
6	27.28±2.32	27.25±2.04	28.33±3.28	28.64±3.01	27.15±3.39

[a] : mean±SD

The increasing rates of body weight of control, 25, 50, 100 and 200ppm $CdCl_2$ administered group for 6 weeks were 27.0%, 28.54%, 28.31%, 20.49% and 18.04%, respectively.

Table 3. Spleen indices, spleen cell number and proportion of CD_4^+ or CD_8^+ cells in spleen of BALB/c mice exposed to $CdCl_2$ in drinking water for 6 weeks[a]

Items	$CdCl_2$ conc(ppm)				
	0	25	50	100	200
Spleen index(mg/g)	4.34±0.23	4.20±0.54	4.80±0.87	4.25±0.32	4.40±0.32
CD_4^+-CD_8^+ cell proportion					
CD_4^+ cell(%)	37.65±3.65	37.42±2.66	36.55±1.87	35.95±1.15	36.40±2.67
CD_8^+ cell(%)	9.52±1.34	8.58±1.11	9.43±1.22	7.95±1.64	7.57±1.04*
CD_4^+/CD_8^+ ratio	3.99±0.32	4.44±0.78	3.92±0.41	4.68±0.88	4.92±0.96
Cell/spleen($\times 10^7$)	24.29±5.98	27.72±5.48	32.96±8.44	28.32±8.76	29.64±4.08
CD_4^+ cell count($\times 10^7$)[b]	9.15±2.24	10.40±2.04	12.04±3.08	10.20±3.16	10.80±1.48
CD_8^+ cell count($\times 10^7$)	2.32±0.56	2.54±0.27	3.12±0.80	2.25±0.70	2.24±0.28
CD_4^+/CD_8^+ ratio	3.97±0.02	4.35±0.01**	3.88±0.01**	4.54±0.03**	4.81±0.03**

[a]: All values represent the mean±SD.

[b]: Numbers of CD_4^+ and CD_8^+ cells were calculated due to CD_4^+ or CD_8^+ cell proportion.

Asterisks denote changes in $CdCl_2$-exposed mice significantly different from control(*P<0.05, **P<0.01).

in all experimental groups. Total CD_4^+ cells in Cd groups were slightly increased as compared with control because of increase in total number of splenocytes, although proportions of CD_4^+ cells(%) in definite number of splenocytes were decreased slightly in all of the Cd fed groups. Proportions of CD_8^+ cells(%) in definite number of splenocytes were decreased in all experimental groups, especially in 200ppm Cd gruops. However, total CD_8^+cells were slightly increased except for 100 and 200ppm Cd groups.

On the other hand, CD_4^+/CD_8^+ ratios in definite number of splenocytes were increased slightly in Cd groups except for 50ppm Cd group as compared with control. The CD_4^+/CD_8^+ ratios in total splenocytes, however, were increased noticeably except for 50ppm Cd group($p<0.001$).

Phagocytosis of peritoneal macrophages : Mice were orally administered various concentrations of Cd for 6 weeks. One day after Cd administration, phagocytosis of peritoneal macrophages against SRBCs were carried out.

As shown Table 4, phagocytosis rates of 25 and 50ppm Cd administration groups were increased significantly as compared with control, but those of 100 and 200ppm Cd administration groups were similar to that of control.

Mitogen-induced splenocyte proliferation : To investigate the maximal responses of splenocyte proliferation against Con-A or LPS, various concentraions of mitogens were added to cultures. From these results, we select concentraions of each mitogen shown peak response of proliferation, 4μg Con-A/ml or 16μg LPS/ml(Fig 2, Fig 3).

On the other hand, various concentrations of $CdCl_2$ (10^{-4}-10^{-7}M) were added to the mitogen-stimulated culture for 48 hours *in vitro*. As shown in Table 5, splenocyte proliferation by LPS was decreased dose dependently, but proliferation by Con-A, T-cell mitogen, was increased slightly in concentrations of 10^{-7}-10^{-6}M $CdCl_2$.

Table 4. Effect of $CdCl_2$ on phagocytosis of peritoneal macrophages

$CdCl_2$ conc(ppm)	% of phagocytosis
0	21.43±3.62
25	36.34±9.45*
50	37.15±9.22**
100	18.20±3.04
200	19.48±3.22

Mice were orally administered various concentrations of $CdCl_2$ for 6 weeks. One day after $CdCl_2$ treatment, 0.5ml of 1% SRBC suspension was injected into the peritoneal cavity of the mouse. Thirty minutes later, peritoneal macrophages were obtained and counted phagocytized cells. Each value represents mean±SD of phagocytosis. Significant difference from the control(*$P<0.05$, **$P<0.01$).

Table 5. Effect of $CdCl_2$ on the proliferation of splenocytes stimulated with Con-A or LPS *in vitro*

$CdCl_2$(log M)	Mitogens[a]	
	Con-A	LPS
0	23342±2392[b]	8428±1257
-7	25599±2618	7328±1316
-6	26007±1045	6727±952
-5	22721±3369	1473±211
-4	2446±440	1205±107

[a] : Indicated concentrations of $CdCl_2$ were added into the culture at the initiation.

[b] : Values were expressed as cpm±SD.

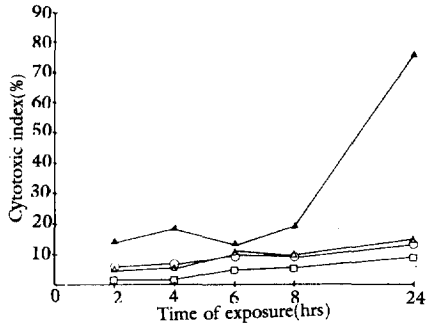

Fig 4. Cytotoxicity of various concentratios of $CdCl_2$ on splenocytes.
Splenocytes(2×10^6 cells/ml) were incubated at 37℃ for the indicated time intervals. The cytotoxicity was measured by trypan blue exclusion test. Each point represents mean from 5 mice. □-□ 10^{-7}M, △-△10^{-6}M, ○-○10^{-5}M, ▲-▲ 10^{-4}M $CdCl_2$

Cytotoxicity : Various concentrations of $CdCl_2$(10^{-4}-10^{-7}M) were added to culture during various times(2, 4, 6, 8 and 24 hrs) for cytotoxicity test *in vitro*.

As shown in Fig 4, significant cytotoxicity of splenocytes by $CdCl_2$ was shown at 10^{-4}M $CdCl_2$ treatment group, especially at 24 hrs. But cytotoxicity of the other $CdCl_2$ concentration group was smiliar to one another.

Discussion

In a recent year, the effects of cadmium on immune responses have been the subject of investigation. However, there are a lot of controversy about the effect of cadmium on immune responses including immunostimulation, immunosuppression and no effect on immune responses[12-14,15-20].

Therefore, in this study, the effects of cadmium on the cellular immune response of BALB/c mice were investigated, when adult male BALB/c mice(6~8 weeks) received distilled water only or water containing 25, 50, 100 or 200ppm Cd for 6 weeks. The results from this study showed that the gain of body weight of cadmium administered mice was no changes in 25 and 50ppm but decreased 100 and 200ppm Cd groups(Table 2). Many authors[11,24,25] reported that various concentrations of Cd administration for several weeks had no significant adverse effect on body weight but other authors[14,18] reported that the body weight gain was lower in Cd administered groups. These findings indicated that changes of body weight induced by cadmium might be affected diversely by dose and time of administration.

In addition, spleen index is useful indicator of immune dysfunction[28]. Mild splenomegaly was observed in 50 and 200ppm Cd groups, although other experimental groups were not shown increase of spleen indices as compared with control(Table 3). Ohsawa[27,29] reported that morphologic shift of small lymphocytes to large lymphocytes as well as lymphopenia and splenomegaly have been most sensitive responses in mice receiving Cd injections, however, other authors[11,22,24] said that no significant differences in spleen weight between Cd-fed and control mice were observed. And also, effect of Cd on total number of splenocytes were controversial including increase[27] or slight decrease[14]. In our reports, total number of splenocytes tended to increase in all Cd-fed groups(Table 3).

Meanwhile, CD_4^+ cells in spleen tended to increase in all experimental groups but CD_8^+ cells were decreased slightly except for 25 and 50ppm Cd groups (Table 3). CD_4^+/CD_8^+ ratios in total splenocytes, however, were increased noticeably except for 50ppm Cd-fed group. These results seem to indicate that the migration of lymphocytes was modified by the exposure to Cd, thereby promoting redistribution of lymphocytes[27]. The mechanism for the differential modification of lymphocyte distribution is not known yet. Ohsawa et al[21,27] reported that high level of plasma glucocorticoid which promoted the distribution of lymphocytes was found in the blood of Cd-injected mice. Therefore, it might induce missing of T and B lymphocytes from blood and their accumulation in spleen. It might be also possible that metal administration caused to produce indirect effects on the lymphoid system due to stress with increased secretion of corticosteroid. Selective lympholytic effects

of corticosteroid could also contribute to the rise in the proportion of cells capable of responding to mitogens and participating in antibody synthesis[14,30].

As for phagocytosis of peritoneal macrophages, some authors[12,24] reported that peritoneal macrophage phagocytosis was significantly increased in Cd treated group, thereby augmenting activity of adherent suppressor cells, but other[31] suggested that Cd increased it or had no effect on it. In this study, it was increased significantly in 25 and 50ppm Cd groups but not affected by 100 and 200ppm Cd(Table 4). These cited reports suggest that there are relationship between activity of peritoneal macrophage and distribution of T-suppressor(CD_8^+) cells in spleen. From the results of this study, it might be a relationship between peritoneal macrophage activity and distribution of splenic CD_8^+ cells.

Previous study had reported that oral exposure of Cd to mice for several weeks enhanced proliferative responses of lymphocyte to various mitogens or caused polyclonal activation of B cell producing antinuclear antibodies(ANA)[11,32]. And also, it might be possible that lymphoid cells responding to Con-A and PHA as well as those participating in the antibody response to SRBC were more resistant than other lymphocyte populations to the toxic effects or Cd accumulation. Thus, their proportion would be increased in the spleen of mice exposed to Cd[14]. We postulated that these enhanced immune responses were induced by increase of T-helper cells in spleen of Cd-fed mice.

Meanwhile, lymphocyte proliferation assay has been used extensively to evaluate activity of lymphocytes *in vitro* because it was similar to proliferative response of antigen exposed immune cells *in vivo*[34], and also used as indicators of exposure to toxic agents[35]. Gaworski and Sharma[25] reported that a significant decrease in the response of splenic lymphocytes to PHA and PWM was found in mice after oral administration of 20 or 160ppm Cd. On the other hand, no decrease in mitogen response was found in splenic lymphocytes of mice that had been maintained on various concentrations of Cd in the drinking water for several weeks[10], whereas Müller et al[23] observed a elevated response of splenic T lymphocytes in Cd-fed mice. Regarding *in vitro* stimulation, it was suggested that Cd and Hg caused to produce dose-dependent inhibition of lymphocyte activities but zinc and lead led to enhancement of lymphocyte proliferation induced by PHA or PWM at a certain level of these heavy metals [25]. These results indicated that the proliferative response of lymphocyte to various mitogens could be showed differently by kinds and concentrations of heavy metals or by experiments such as *in vitro* and *in vivo*. In this study, proliferative response to the T cell mitogen, Con-A, was increased slightly in the range of 10^{-7}-10^{-6}M $CdCl_2$, but to the B cell mitogen, LPS, it was decreased dose dependently(Table 5). The enhanced responses induced by low concentrations of Cd could be explained by several reports that these low concentrations of Cd would induce activation of T cell which might be more resistant to Cd toxicity[14] and have mitogenic effect[21,32,36].

The result of this study suggested that Cd would be able to modify the distribution of T cell subpopulations which could play a major role in the cellular immune responses.

Finally, it could be concluded that Cd might modulate the cellular immune response under circumstances. However further study is needed to correlate Cd metabolism and distribution with immunotoxicity.

Summary

This study was undertaken to investigate the effects of Cd administered *ad libitum* for 6 weeks on the cellular immune responses of Balb/c mice. The results were summarized as follows;

1. The mice fed 25, 50 and 100ppm Cd drank as much as control, but the mice fed 200ppm Cd drank significantly less water after Cd exposure than did control. Increasing rates of body weight of Cd-fed mice for 6 weeks were as this, control group 27.0%, Cd administered groups(25, 50, 100 and 200ppm) 28.54%, 28.31%, 20.49% and 18.04%, respectively.

2. Absolute spleen to body weight(mg/g) of con-

trol, 25, 50, 100 and 200ppm Cd administered groups were 4.34±0.23, 4.20±0.54, 4.80±0.87, 4.25±0.32 and 4.40±0.32, respectively. Splenic cellularity($\times 10^7$) of control was 24.29±5.98 but increased to 27.72±5.48, 32.96±8.44, 28.32±8.76 and 29.64±4.08 in 25, 50, 100 and 200ppm Cd-fed groups, respectively.

3. Total CD_4^+ cells($\times 10^7$) of control, 25, 50, 100 and 200ppm Cd-fed groups were 9.15±2.24, 10.40±2.04, 12.04±3.08, 10.20±3.16 and 10.80±1.48, respectively and total CD_8^+ cells($\times 10^7$) of these groups were 2.32±0.56, 2.54±0.27, 3.12±0.80, 2.25±0.70 and 2.24±0.28, in order. On the other hand, CD_4^+/CD_8^+ ratios in total cells were increased significantly except for 50ppm Cd-fed group(3.88±0.01). And that of control was 3.97±0.02, but those of 25, 100 and 200ppm were 4.35±0.01, 4.54±0.03 and 4.81±0.03.

4. Phagocytosis rates of peritoneal macrophages were increased significantly in 25 and 50ppm Cd groups(36.34±9.45 and 37.15±9.22, respectively), but 100 and 200ppm groups showed similar rates(18.20±3.04 and 19.48±3.22, respectively) to that of control(21.43±3.62).

5. In mitogen-induced splenocyte proliferation, various concentraions of $CdCl_2$(10^{-4}-10^{-7}M) were added to mitogen-stimulated culture *in vitro*. Splenocyte proliferation induced by LPS was decreased dose dependently, but proliferation by Con-A was increased slightly in concentrations of 10^{-7}-10^{-6}M.

6. Significant cytotoxicity of splenocytes with $CdCl_2$ were shown at 10^{-4}M treated group, especially at 24 hrs.

From these results, it could be concluded that Cd might modulate the immune responses by modifying a distribution of T cell subpopulations.

References

1. Marcus MK, Austin FH, Jack B, et al. *Occupational diseases*. Revised edition 1977; 345-348.
2. John NH. *Nutritional toxicology*. London: Academic Press, 1982; 164-173.
3. Frank EG, Jerome JP. *Introduction to environmental toxicology*. New York; Elsevier, 1980; 31-42.
4. Goyer RA. Toxic effects of metals. In: Klaassem CD, Amdur MO, Doull J(eds), *Casarett and Doull's Toxicology*. 3rd ed, New York: Macmillian Publishing co, 1986; 592-596.
5. Yost KJ. Some aspects of cadmium flow in the US *Environ Health Perspect* 1979; 28: 5-16.
6. Kostic MM, Ognjanovic B, Dimitrijevic S, et al. Cadmium-induced changes of antioxidant and metabolic status in red blood cells of rat *in vivo* effects. *Eur J Haematol* 1993; 51: 86-92.
7. Dudley RE, Svovoda DJ, Klassen CD. Acute exposure to cadmium causes severe liver injury in rats. *Toxicol Appl Pharmacol* 1982; 65: 302-313.
8. Pond WG, Walker EF, Kirtland D. Cadmium induced anemia in growing pig. Protective effect of oral or parenteral iron. *J Nutr* 1973; 36: 1122-1124.
9. Perry HM, Erlanger A, Yunice SE, et al. Hypertension and tissue matal levels following intravenous cadmium, mercury, and zinc. *Am J Physiol* 1970; 219: 755-761.
10. Koller LD, Roan JG, Kerkvliet NI. Mitogen stimulation of lymphocytes in CBA mice exposed to lead and cadmium. *Environ Res* 1979; 19: 177-188.
11. Ohsawa M, Takahashi K, Otsuka F. Induction of anti-nuclear antibodies in mice orally exposed to cadmium at low concentrations. *Clin Exp Immunol* 1988; 73: 98-102.
12. Koller LD, Roan JG. Effects of lead and cadmium on mouse peritoneal macrophages. *J Reticuloendothel Soc* 1977; 21: 7-12.
13. Fujimaki H, Murakami M, Kubota K. *In vitro* evaluation of cadmium induced augmentation of the antibody response. *Toxicol Appl Pharmacol* 1982; 62: 288-293.
14. Malave I, De Ruffino D. Altered immune response during cadmium administration in mice. *Toxicol Appl Pharmacol* 1984; 74: 46-56.
15. Graham J, Miller F, Daniels PE, et al. Influence of cadmium, nickel and chromium on primary immunity in mice. *Environ Res* 1978; 16: 77-87.
16. Koller LD, Exon JH, Roan JG. Antibody suppres-

sion by cadmium. *Arch Environ Health* 1975; 30: 598-601.

17. Lawrence DL. Heavy metal modulation of lymphocyte activities. I. *in vitro* effects of heavy metals on primary humoral immune responses. *Toxicol Appl Pharmacol* 1981; 57: 439-451.
18. Koller LD. Immunosuppression produced by lead, cadmium and mercury. *Am J Vet Res* 1973; 34: 1457-1458.
19. Stelzer KJ, Pazdernik TL. Cadmium-induced immunotoxicity. *Int J Immunopharmacol* 1983; 5: 541-548.
20. Wesenberg GBR, Wesenberg F. Effect of cadmium on the immune response in rats. *Environ Res* 1983; 31: 413-419.
21. Ohsawa M, Sato KM, Takahashi K, et al. Strain differences in cadmium-mediated suppression of lymphocyte proliferation in mice. *Toxicol Appl Pharmacol* 1986; 84: 379-388.
22. Ohsawa M. Immunotoxicity of cadmium. In: Hashimoto K and Minami M, et al. *Seminars of toxicity mechanisms-1,* center for Academic Publications Tokyo. 1987; 57-63.
23. Müller S, Gillert KE, Krause C, et al. Effects of cadmium on the immune system of mice. *Experientia* 1978; 35: 909-910.
24. Thomas PT, Ratajczak HV, Aranyi C, et al. Evaluation of host resistance and immune function in cadmium-exposed mice. *Toxicol Appl Pharmacol* 1985; 80: 446-456.
25. Gaworski CL, Sharma RP. The effects of heavy metals on [^{3}H] thymidine uptake in lymphocyte. *Toxicol Appl Pharmacol* 1978; 46: 305-313.
26. Wesenberg G, Frosse G, Rasmussen P, et al. Cadmium content of indicatior and target organs in rats after graded doses of cadmium. *Int J Environ Stud* 1981; 16: 147-155.
27. Ohsawa M, Sato K, Takahashi K, et al. Modified distribution of lymphocyte subpopulations in blood and spleen from mice exposed to cadmium. *Toxicol lett* 1983; 19: 29-35.
28. Hayes AW. Immune system: evaluation on injury in principles and methods of toxicology. 2nd ed. New York: Raven Press, 1989; 741-760.
29. Ohsawa M, Kawai K. Cytological shift in lymphocytes induced by cadmium in mice and rat. *Environ Res* 1981; 24: 192-200.
30. Khansari DN, Murgo AJ, Faith RE. Effects of stress on the immune system. *Immunol Today* 1990; 11: 170-175.
31. Ohsawa M. Nutritional and toxicological implication of trace elements in the immune response. *Essential and Toxic Trace Elements in Human Health and Disease*, Wiley-Liss, 1993; 283-298.
32. Shenker BJ, Matarazzo WJ, Hirsch RL, et al. Trace metal modification of immunocompetence. I. Effect of trace metal in the cultures on *in vitro* transformation of B lymphocytes. *Cell Immunol* 1977; 34: 19-24.
33. Jones RH, Williams RL, Jones AM. Effects of heavy metal on the immune response. Preliminary findings for cadmium in rats. *Proc Soc Exp Biol Med* 1971; 137: 1231-1236.
34. Catalona WJ, Tarpley JL, Potvin C, et al. Correlations among cutaneous reactivity to DNCB, PHA-induced lymphocyte blastogenesis and peripheral blood E rosettes. *Clin Exp Immunol* 1975; 19: 327-332.
35. Snyder CA, Valle CD. Lymphocyte proliferation assays as potential biomarkers for toxicant exposures. *J Toxicol Environ Health* 1991; 34: 127-139.
36. Gallagher K, Matarazzo WJ, Gray I. Trace metal modification of immunocompetence. II. Effect of Pb^{2+}, Cd^{2+} and Cr^{3+} on RNA turnover, hexokinase activity, and blastogenesis during B-lymphocyte transformation *in vitro*. *Clin Immunol Immunopathol* 1979; 13: 369-377.
37. Pelletier L, Pasquier R, Rossert J, et al. $HgCl_2$ induces nonspecific immunosuppression in Lewis rats. *Eur J Immunol* 1987; 17: 49-54.
38. David WO, Shen ML, Luo YD. Effects of cocaine on the immune system on BALB/c mice. *Clin Immunol Immunopathol* 1989; 52: 305-312.