The Effect of Cadmium Chloride on the Immune Response in Mice

B.R. Blakley*

ABSTRACT

Six week old BDF_1 female mice were exposed to cadmium chloride in the drinking water at concentrations ranging from 0 to 50 μg/mL cadmium for three weeks. The humoral immune response against sheep red blood cells which is T-lymphocyte and macrophage dependent, was suppressed in a dose dependent fashion with the maximum suppression of 28.2% observed in the highest exposure group ($P < 0.0001$). Mitogen studies demonstrated that cadmium was a weak mitogen, producing a dose-dependent enhancement of blastogenesis ($P = 0.026$). T-lymphocyte responses which were induced by concanavalin A were not affected by cadmium exposure ($P = 0.284$). A dose-dependent enhancement of the B-lymphocyte activity was produced in the presence of cadmium when the lymphocytes were induced with *Escherichia coli*, lipopolysaccharide, a B-lymphocyte mitogen ($P = 0.007$). These results suggest that the immunosuppressive effects of cadmium associated with the humoral immune response are not due to an impairment of lymphocyte proliferation, an intermediate step involved in the generation of an immune response. The immunosuppressive effects were produced at relatively low cadmium exposures as indicated by the renal cadmium concentrations suggesting that the immune system is very vulnerable to the toxic effects of cadmium.

Key words: cadmium, immunotoxicology, immunosuppression, humoral immume response, lymphocyte.

RÉSUMÉ

Cette expérience s'échelonnait sur une période de trois semaines et elle consistait à ajouter jusqu'à 50 μg/mL de chlorure de cadmium dans l'eau de boisson de souris BDF, femelles et âgées de six semaines. L'intervention supprima la réaction immunologique humorale envers les hématies de moutons, réaction subordonnée aux lymphocytes T et aux macrophages. Cette suppression s'avéra proportionnelle à la dose de chlorure de cadmium et elle atteignit son point culminant, i.e. 28,2%, chez le groupe de souris auquel on donnait la plus forte dose ($P < 0,0001$). Une analyse appropriée révéla que le cadmium est un piètre mitogène qui stimule la blastogènèse, proportionnellement à sa concentration ($P = 0,026$). Les réactions des lymphocytes T, déclenchées par la concanavaline A, ne subirent aucune influence de la part du cadmium ($P = 0,284$). Une intensification de l'activité des lymphocytes B, reliée à la dose de cadmium, se produisit sous l'influence du lipopolysaccharide d'*Escherichia coli*, un mitogène des lymphocytes B ($P = 0,007$). Les résultats de cette expérience laissent supposer que les effets immunosuppresseurs du cadmium qui accompagnent la réaction immunologique humorale ne résultent pas d'une altération de la prolifération des lymphocytes, une étape intermédiaire dans la génération d'une réaction immunitaire. Les effets immunosuppresseurs se produisirent à des doses relativement faibles de cadmium, comme la démontrèrent les concentrations rénales de cadmium, indice que le système immunitaire est très vulnérable aux effets toxiques du cadmium.

Mots clés: cadmium, immunotoxicologie, immunosuppression, réaction immunitaire humorale, lymphocyte.

INTRODUCTION

Environmental toxicants such as cadmium may produce a variety of clinical manifestations. In man and animals, several organ systems including the renal, hepatic, respiratory and vasculature systems may be affected by cadmium exposure (1,2,3,4). In addition, prolonged low level exposure to cadmium can alter the immune responses. Several studies have documented the effects of cadium on the immune system. Cadmium exposure enhances the susceptibility to bacterial (5), viral (6) and protozoal infections (7). Primary and secondary immune responses against specific antigens such as sheep red blood cells (SRBC) (8,9,10) or antibody titres against infectious agents (11) were also suppressed by cadmium. Cell mediated responses such as the delayed-type hypersensitivity reaction against SRBC were also suppressed (12). Subtle changes in the mitogen responsiveness of lymphocytes (13,14,15) or shifts in lymphocytes subpopulations (16) subsequent to cadmium exposure may also reflect an altered immune status. Other cellular components such as the macrophage which are involved in the generation of an immune response are adversely affected by cadmium (17,18). A reduction in the release of soluble cell products such as lymphokines which are necessary for an optimal immune response also occurs following cadmium exposure (19).

In the present study, the effects of *in vivo* cadmium exposure on T-cell dependent antibody production against SRBC and T- and B-lymphocyte mitogen responses in mice were investigated at several different levels of cadmium exposure.

*Department of Veterinary Physiological Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0.

Submitted March 13, 1984.

MATERIALS AND METHODS

Six week old BDF_1 female mice (Jackson Laboratory, Bar Harbor, Maine) were acclimatized for one week before exposure to cadmium chloride in deionized distilled drinking water at concentrations ranging from 5 to 50 μg/mL cadmium. After a three week exposure period, the immune status of each mouse was assessed. Weight gains were monitored during the exposure period.

MITOGEN STUDIES

The response of splenic lymphocytes to specific lymphocyte mitogens was evaluated in the cadmium-exposed mice using previously established methods (20). Following cervical dislocation, each mouse was washed with 95% ethanol. The spleen was removed using sterile technique and placed in about 1 mL of cold Eagle's minimum essential medium with L-glutamine, 0.5 mM (Grand Island Biological Co., Grand Island, New York), containing gentamycin sulfate, 10 mg/L (Schering Canada Inc., Pointe Claire, Quebec), at a pH of 7.2. The spleen cells were teased from the splenic connective tissue using sponge forceps. Cellular debris was removed and spleen cell suspensions containing 1 x 10^6 cells/mL were prepared in RPMI 1640 containing L-glutamine, 0.5 mM, 10% (v/v) filtered fetal calf serum (Grand Island Biological Co., Grand Island, New York) and gentamycin sulfate (10 mg/L). The cell viability was determined using the trypan blue dye exclusion technique.

The spleen cell suspensions from individual mice were placed in quadruplicate into 96-well round bottom microtiter plates (0.2 mL/well). Optimal amounts of specific lymphocyte mitogens, concanavalin A (Con A), 0.5 μg (Sigma Chemical Co., St. Louis, Missouri) or lipopolysaccharide B *Escherichia coli* 026:B6 (LPS), 10 μg (Difco Laboratories, Detroit, Michigan) were added in 10 μL volumes to the cell suspensions for *in vitro* lymphocyte stimulation. The optimal amounts of each lymphocyte mitogen were determined in preliminary studies using lymphocytes from unexposed mice and four different concentrations of each mitogen. Background responses were obtained from spleen cell suspensions containing no mitogen. The microtiter plates were incubated at 37°C in a humidified environment containing 7% O_2, 10% CO_2 and 83% N_2. After a 42 hour incubation period, 0.5 μCi of [^{3}H] thymidine (methyl-^{3}H, 6.7 Ci, mmol) (New England Nuclear, Boston, Massachusetts) was added to each cell suspension. Six hours later, the cell suspensions were harvested on a 24 well microharvester (Bellco Glass Inc., Vineland, New Jersey). The cells were collected on glass-fibre filters, washed three times with saline and once with 95% ethanol. The filters were placed in 7 mL scintillation vials containing 3 mL of scintillation fluid (Ready Solv HP/b, Beckman Instruments Inc., Fullerton, California) and counted on a Searle Analytic Isocap/300 liquid scintillation spectrometer.

PLAQUE FORMING CELL STUDIES

The humoral immune response was assessed using the Jerne plaque assay (21). Following the three week cadmium exposure period, an antigenic stimulus was administered to each mouse. The primary immune response was measured five days later. Sheep red blood cells (SRBC) which were used as the antigenic stimulus were administered ip in a 10% suspension (0.25 mL/mouse). Spleen cell suspensions prepared in a fashion similar to the mitogen studies containing 3 x 10^6 cells/mL were utilized in the plaque assay. Background plaque counts were obtained from mice administered 0.25 mL of saline rather than the SRBC suspension. Components of a single assay included: 20 μL of SRBC (25% suspension in Hank's BS containing 5% fetal calf serum), 20 μL of guinea pig complement, 60 μL of Hank's BS containing 5% fetal calf serum (all reagents: Grand Island Biological Co., Grand Island, New York) and 100 μL of spleen cell suspension. The mixture was incubated at 37°C for one-half hour as a monolayer suspension to facilitate plaque production. Each plaque or area of SRBC-lysis indicated the presence of a specific antibody-producing cell (anti-SRBC). The plaque production was enumerated visually under fluorescent light against a dark background.

TISSUE ANALYSIS

Representative samples of kidney from mice exposed to cadmium were saved for cadmium analysis. The kidneys were digested in acid-cleaned glassware using 10 mL of a 5:1 nitric: perchloric acid mixture. The digest was heated until dense white perchloric and fumes appeared. This residue was more completely digested using 3 mL of a 1:1 nitric:sulfuric acid mixture. The cooled acid residue was diluted to 10 mL with double-distilled, deionized water and analyzed using atomic absorption spectrophotometry.

STATISTICAL ANALYSIS

Analysis of variance and linear regression including slope and intercept comparisons were used for statistical description of the data and to determine the presence of treatment effects (22,23).

RESULTS

In this study, mice which were exposed to cadmium in the drinking water at concentrations as high as 50 μg/mL showed no evidence of overt clinical toxicity. Weight gains during the three week exposure period were not affected by cadmium (Table I). No gross pathological lesions associated with cadmium exposure were observed in the mice following cervical dislocation and dissection. The mice appeared to tolerate these cadmium exposures without difficulty.

TISSUE CONCENTRATIONS OF CADMIUM

Whole kidney concentrations of cadmium which were determined after the three week exposure period are summarized in Table II. A dose-dependent increase in the cadmium concentration of the kidney was observed. The cadmium concentrations in the kidney are similar to those reported in other studies (8,14) utilizing comparable cadmium-exposure regimens.

PLAQUE-FORMING CELL STUDIES

The primary immune response against SRBC (anti-SRBC plaque-forming cells) was suppressed by cadmium exposure. Table III indicates

TABLE I. Weight Gains in Female BDF_1 Mice Exposed to Cadmium Chloride for Three Weeks

Cadmium concentration in the drinking water (μg mL)	Weight gain (g mouse day)	Probability
0	0.087 ± 0.009[a] (22)[b]	0.929[c]
5	0.081 ± 0.010 (22)	
10	0.090 ± 0.010 (22)	
50	0.083 ± 0.010 (22)	

[a]Mean ± SE
[b]Number of animals
[c]Probability of no cadmium treatment difference

TABLE II. Concentration of Cadmium in the Kidney of Female BDF_1 Mice Exposed to Cadmium Chloride for Three Weeks

Cadmium concentration in the drinking water (μg mL)	Whole kidney cadmium concentration[a] (nmole g)	Probability
0	1.51 ± 0.54[b] (10)[c]	< 0.0001[d]
5	2.95 ± 1.16 (10)	
10	9.11 ± 1.34 (10)	
50	53.21 ± 5.27 (10)	

[a]Wet weight
[b]Mean ± SE
[c]Number of tissues (animals)
[d]Probability of no cadmium treatment difference

TABLE III. The Effect of Cadmium Chloride[a] on the Primary Immune Response[b] in Female BDF_1 Mice

Cadmium concentration in the drinking water (μg mL)	Plaque-forming cells[c] per 10^6 spleen cells	Percentage of control	Probability
0	411 ± 33[d] (11)[e]	100	< 0.0001[f]
5	346 ± 26 (11)	84.2	
10	337 ± 26 (11)	82.0	
50	295 ± 26 (10)	71.8	

[a]Cadmium chloride exposure 26 days
[b]IgM or 19s antibody response against sheep red blood cells following antigenic challenge on day 21 of cadmium exposure
[c]Antibody-producing cells
[d]Mean ± SE
[e]Number of animals
[f]Probability of no cadmium treatment differences

TABLE IV. The Effects of Cadmium Chloride[a] on the Induction of DNA Synthesis[b] by the Lymphocyte Mitogens[c]

Cadmium concentration in the drinking water (μg mL)	Counts per Minute		
	control (no mitogen)	LPS	Con A
0	73 ± 12[d] (9)[e]	1436 ± 239 (9)	9442 ± 1003 (9)
5	64 ± 8 (9)	1185 ± 181 (9)	9682 ± 1117 (9)
10	107 ± 14 (10)	2569 ± 433 (10)	13219 ± 1737 (10)
50	96 ± 13 (10)	2906 ± 751 (10)	13688 ± 1479 (10)
Probability[f]	0.026	0.007	0.110

[a]Female BDF_1 mice exposed to cadmium chloride for three weeks
[b]Incorporation of [^{3}H] thymidine
[c]Lymphocyte mitogens — *E. coli* lipopolysaccharide B 026:B6 (LPS), concanavalin A (Con A)
[d]Mean ± SE
[e]Number of mice in each group, tested in triplicate
[f]Probability of no cadmium treatment differences using a one way analysis of variance on log transformed data

that cadmium exposure produces a dose-dependent suppression of the primary immune response. Immunosuppression was observed at all cadmium exposures, with the maximum suppression of 28.2% observed in the highest exposure group.

MITOGEN STUDIES

The induction of DNA synthesis by lymphocyte mitogens was not suppressed by *in vivo* cadmium exposure. The effects of cadmium on the specific T-lymphocyte or B-lymphocyte mitogen responses are summarized in Table IV. Cadmium exposure produces a dose-dependent enhancement of DNA synthesis induced by LPS, a B-lymphocyte mitogen. A similar enhancement but to a lesser extent was also seen in lymphocytes that were exposed only to cadmium but no mitogen. This indicates that cadmium is a weak mitogen. The mitogen responses induced by Con A, a T-lymphocyte mitogen, were not affected by cadmium exposure, though a similar dose-dependent enhancement comparable to the no mitogen responses was observed. The mathematical relationship describing the association between cadmium exposure and each mitogen response is summarized in Table V. Using linear regression analysis, the dose-related response in each instance was described. The positive slope in each case reflects the weak mitogenic activity of cadmium and in the case of the LPS studies, it also reflects enhanced B-lymphocyte cell division. Statistical comparison of the slopes and intercepts (23) derived from the linear regression equations is tabulated in Table VI. Statistically, all of

TABLE V. Statistical Analysis of the Effect of Cadmium Chloride on the Induction of DNA Synthesis by Lymphocyte Mitogens[a] using Linear Regression[b]

	Control No Mitogen	LPS	Con A
Intercept (b)	1.86[c]	3.12	3.98
Regression coefficient (m)	0.0022[d]	0.0055	0.0029

[a]Lymphocyte mitogens — *E. coli* lipopolysaccharide B 026:B6 (LPS), concanavalin A (Con A)
[b]Linear regression equation = log counts per minute = b + m (cadmium concentration μg/mL)
[c]Counts per minute (log transformed)
[d]Slope of regression line (log transformed)

TABLE VI. Comparison of the Slopes and Intercepts Derived from Linear Regression Equations[a] of DNA Synthesis Induction by Lymphocyte Mitogens[b] following Cadmium Exposure[c]

Comparison	Probability of No Treatment Difference	
	LPS	Con A
Slope (m)		
No mitogen	0.0002	0.284
LPS	—	0.022
Intercept (b)		
No mitogen	< 0.0001	< 0.0001
LPS	—	< 0.0001

[a]Linear regression analysis performed on log transformed data

[b]Lymphocyte mitogens — *E. coli* lipopolysaccharide B 026:B6 (LPS), concanavalin A (Con A)

[c]Female BDF_1 mice exposed to cadmium chloride for three weeks

the intercepts are different from each other. These differences are not due to cadmium exposure but rather reflect the different capacities of each mitogen, Con A, LPS, or no mitogen, to induce DNA synthesis required for cell division. Comparison of the slopes obtained from the Con A and no mitogen responses indicates that the slopes are similar. The enhancement which was observed in both instances is due only to the weak mitogenic activity of cadmium. T-lymphocytes are not specifically affected by cadmium. In contrast, comparisons of the slopes obtained from LPS and Con A or no mitogen studies indicate that cadmium greatly enhances the mitogen response to LPS, a B-lymphocyte mitogen. The enhancement which was observed exceeds the response which would be expected if only the weak mitogenic properties of cadmium were present. Cadmium does cause a specific enhancement of B-lymphocyte mitogen responses.

DISCUSSION

A dose-dependent suppression of the humoral immune response against SRBC was observed in mice exposed to cadmium. Similar observations have been reported by other investigators using a variety of different cadmium-exposure regimens. Both primary and secondary immune responses against SRBC were suppressed (8,9,10). Antigens such as SRBC are T-lymphocyte dependent and require the presence of macrophages before an antibody response is generated by the B-lymphocyte (24). Functional deficits in B-lymphocyte, T-lymphocyte, macrophage activity or any combination thereof may suppress a T-lymphocyte, macrophage dependent antibody response. Cadmium may be producing a specific or a generalized cellular alteration. Many studies have examined specific cell populations in the presence of cadmium.

Specific cell populations which were isolated from normal experimental animals have been exposed to cadmium *in vitro*. Dose-response information pertaining to various components of the immune system have been examined. *In vitro* cadmium exposure does suppress the antibody response against SRBC in a fashion comparable to the *in vivo* cadmium exposure studies (25). Macrophage functions including phagocytosis (18), cytolytic activity against tumor cells (17) and enzymatic activity (17) are reduced significantly by cadmium. Classical cell mediated immune responses such as delayed type hypersensitivity reactions are also suppressed by cadmium (12,26).

Caution should be exercised in interpretation of the effects of *in vitro* cadmium exposure. In several studies (17,19,25,27), *in vitro* cadmium exposure of about 1×10^{-4} M cadmium causes a generalized cytotoxic effect resulting in a reduction of lymphocyte and macrophage activity. The evaluation of functional deficits in specific cell populations can only be realistically evaluated at lower exposures that more closely resemble the *in vivo* conditions. High *in vitro* cadmium exposure may provide useful information to suggest which cell populations or cell functions need to be examined utilizing *in vivo* exposure conditions. *In vivo*, extensive cellular destruction such as renal tubular necrosis which may correspond to the *in vitro* cytotoxic effects occurs when the kidney cadmium concentration approaches 1.79 μmol/g (28). The functional alterations in the immune system in this and similar studies (8,14) however, were associated with substantially lower renal cadmium concentrations.

Mitogen responses of lymphocytes in the present study suggest that cadmium produces a weak mitogenic response in both T- and B-lymphocytes. In the presence of specific lymphocyte mitogens, cadmium produced no net effect on T-lymphocytes, but did produce a significant dose-dependent enhancement of B-lymphocyte responses. Similar observations have been reported by other investigators. In the presence of cadmium, enhanced responses to LPS, a B-lymphocyte mitogen have been seen (12,14,15). Chronic cadmium exposure produces cytological shifts in lymphocyte populations (16) which may be due to selective stimulation of specific lymphocyte subpopulations. This observation appears to be consistent with the observed enhancement of B-lymphocyte activity seen in the present mitogen studies.

Similar studies investigating T-lymphocyte mitogen responses indicate a slight enhanced effect or no significant effect following cadmium exposure, though in most instances, no adjustment was made for the weak nonspecific mitogenic activity of cadmium (12,14,15,29). Studies that investigated the role of the macrophage in the mitogen response determined that similar B- or T-lymphocyte responses in the presence of cadmium were produced in the presence or absence of macrophages (14,15). This suggests that the macrophage may not be playing a major role in these mitogen responses. A deficit in macrophage function would not be easily detected using mitogen studies.

Amplification and expression of an immune response can be considered a series of steps or processes involving various cellular components and their interactions. Features including antigen contact, processing and recognition, cellular and clonal proliferation and the final effect or action are examples of these processes (30). Lymphocyte responses to mitogens are an example of clonal proliferation. In the present study, the normal or enhanced lymphocyte responses to mitogens indicates that cadmium does not impair lymphocyte proliferation processes. It has been suggested that cadmium suppresses the SRBC antibody responses, a response requiring macrophage, T- and B-lymphocyte interactions by disrupting an early cellular

event. A reduction of antigen recognition by macrophages and an impairment of optimal cell-cell contact have been suggested as potential mechanisms of action (17), though deficits in other components of the humoral immune pathway may also be present.

ACKNOWLEDGMENTS

The author wishes to acknowledge the technical assistance provided by Mrs. C. Coghlin. This research was supported by the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

1. **ADAMS RG, HARRISON JF, SCOTT P.** The development of cadmium-induced proteinuria, impaired renal function and osteomalacia in aklaline battery workers. Quart J Med 1969; 38: 425-443.
2. **AXELSSON B, PISCATOR M.** Renal damage after prolonged exposure to cadmium: an experimental study. Arch Environ Health 1966; 12: 360-373.
3. **STOWE HD, WILSON M, GOYER RA.** Clinical and morphologic effects of oral cadmium toxicity in rabbits. Arch Pathol 1972; 94: 389-405.
4. **PERRY HM, THIND GS, PERRY EF.** The biology of cadmium. Med Clin N Am 1976; 60: 759-769.
5. **COOK JA, HOFFMAN EO, DI LUZIO NR.** Influence of lead and cadmium on the susceptibility of rats to bacterial challenge. Proc Soc Exp Biol Med 1975; 150: 741-747.
6. **GAINER JH.** Effects of heavy metals and of deficiency of zinc on mortality rates in mice infected with encephalomyocarditis virus. Am J Vet Res 1977; 38: 869-872.
7. **EXON JH, PATTON NW, KOLLER LD.** Hexamitiasis in cadmium-exposed mice. Arch Environ Health 1975; 30: 463-464.
8. **KOLLER LD, EXON JH, ROAN JG.** Antibody suppression by cadmium. Arch Environ Health 1975; 30: 598-601.
9. **KOLLER LD, EXON JH, ROAN JG.** Humoral antibody response in mice after single dose exposure to lead or cadmium. Proc Soc Exp Biol Med 1976; 151: 339-342.
10. **BOZELKA BE, BURKHOLDER P, CHANG L.** Cadmium: a metallic inhibitor of antibody-mediated immunity in mice. Environ Res 1978; 17: 390-402.
11. **KOLLER LD.** Immunosuppression produced by lead, cadmium and mercury. Am J Vet Res 1973; 34: 1457-1458.
12. **MULLER S, GILLERT KE, KRAUSE C, JAUTZKE G, GROSS U, DIAMANTSTEIN T.** Effects of cadmium on the immune system of mice. Experientia 1979; 35: 909-910.
13. **KOLLER LD, ROAN JG.** Response of lymphocytes from lead, cadmium and methylmercury exposed mice in the mixed lymphocyte culture. J Environ Pathol Toxicol 1980; 4: 393-398.
14. **KOLLER LD, ROAN JG, ISAACSON-KERKVLIET N.** Mitogen stimulation of lymphocytes in CBA mice exposed to lead and cadmium. Environ Res 1979; 19: 177-188.
15. **KOLLER LD, ROAN JG, ISAACSON-KERKVLIET N.** Evaluation of data from mitogen studies in CBA mice: comparison of counts per minute, stimulation index and relative proliferation index. Am J Vet Res 1979; 40: 863-866.
16. **OHSAWA M, KAWAI K.** Cytological shift in lymphocytes induced by cadmium in mice and rats. Environ Res 1981; 24: 192-200.
17. **NELSON DJ, KIREMIDJIAN-SCHUMACHER L, STOTZKY G.** Effects of cadmium, lead and zinc on macrophage-mediated cytotoxicity toward tumor cells. Environ Res 1982; 28: 154-163.
18. **LOOSE LD, SILKWORTH JB, WARRINGTON D.** Cadmium-induced phagocyte cytotoxicity. Bull Environ Contam Toxicol 1978; 20: 582-588.
19. **KIREMIDJIAN-SCHUMACHER L, STOTZKY G, LIKHITE V, SCHWARTZ J, DICKSTEIN RA.** Influence of cadmium lead and zinc on the ability of sensitized guinea pig lymphocytes to interact with specific antigen and to produce lymphokine. Environ Res 1981; 24: 96-105.
20. **BLAKLEY BR, ARCHER DL.** Mitogen stimulation of lymphocytes exposed to lead. Toxicol Appl Pharmacol 1982; 62: 183-189.
21. **JERNE NK, HENRY C, NORDIN AA, FUJI H, KOROS MC, LEFKOVITS I.** Plaque forming cells: methodology and theory. Transplant Rev 1974; 18: 130-191.
22. **OSTLE B, MENSING R.** Statistics in research. 3rd ed. Ames: Iowa State University Press, 1975.
23. **KLEINBAUM DG, KUPPER LL.** Applied regression analysis and other multivariable methods. Chapter 8. North Scituate, Massachusetts: Duxbury Press, 1978: 95-112.
24. **FELDMAN M, BASTEN A.** Cell interactions in the immune response *in vitro*. IV. Comparison of the effects of antigen-specific and allogenic thymus-derived cell factors. J Exp Med 1972; 136: 722-736.
25. **LAWRENCE DA.** 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.
26. **FUJIMAKI H, SHIMIZU F, KAWAMURA R, KUBOTA K.** Inhibition of delayed hypersensitivity reaction in mice by cadmium. Toxicol Lett 1983; 19: 241-245.
27. **SHENKER BJ, MATARAZZO WJ, HIRSCH RL, GRAY I.** Trace metal modification of immunocompetence. I. Effect of trace metals in the cultures on *in vitro* transformation of B-lymphocytes. Cell Immunol 1977; 34: 19-24.
28. **FRIBERG L, PISCATOR M, NORDBERG G.** Cadmium in the environment. Cleveland, Ohio: CRC Press, 1971.
29. **RAMSTEN-WESENBERG GB, WESENBERG F.** Effect of cadmium on the immune response in rats. Environ Res 1983; 31: 413-419.
30. **SHARMA RP, ZEEMAN MG.** Immunologic alterations by environmental chemicals: relevance of studying mechanisms versus effects. J Immunopharmacol 1980; 2: 285-307.