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Immunologic Findings Among Lead-Exposed Workers

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A comprehensive panel of immune parameters was evaluated among 145 lead-exposed workers with a median blood lead level (BLL) of 39 μg/dL (range: 15-55 μg/dL) and 84 unexposed workers. After adjusting for covariates, we found no major differences in the percentage of CD3+ cells, CD4+ T cells, CD8+ T cells, B cells, or NK cells between lead-exposed and unexposed workers, although the association between lead exposure and the number of CD4+ T cells was modified by age. We also found no differences between exposed and unexposed workers in serum immunoglobulin levels, salivary IgA, C3 complement levels, or lymphoproliferative responses. However, among exposed workers, the percentage and number of B cells were positively associated with current BLL, serum IgG was negatively associated with cumulative lead exposure, and the percentage and number of CD4+/CD45RA+ cells were positively associated with cumulative lead exposure. We found no evidence of a marked immunotoxic effect of lead at the exposure levels studied, although some subtle differences in immunologic parameters were noted. Am. J. Ind. Med. 33:400–408, 1998. *© 1998 Wiley-Liss, Inc.*†

KEY WORDS: lead; immune system; CD4+ T lymphocyte; immunoglobulins

INTRODUCTION

Although much progress has been made in reducing lead exposure, exposures in the workplace continue to be a significant public health problem. Thousands of adult elevated blood lead levels (BLLs) ≥ 25 μg/dL are reported each year to the National Institute for Occupational Safety and Health (NIOSH) by states participating in the NIOSH Adult Blood Lead Epidemiology and Surveillance (ABLES) programs [CDC, 1996]. Data from phase I of the third National Health and Nutrition Examination Survey indicate that 700,000 adults in the United States may have BLLs ≥ 5 μg/dL [Brody et al., 1994]. Scientific understanding of the wide range of effects associated with lead exposure, including subclinical effects from low level exposure, continues to evolve. Effects of lead have been reported for many organ systems, including the central nervous system, hematologic system, and reproductive system.

Exposure to lead also has been shown to impair immune function. Numerous studies have been conducted in experimental animals to characterize and determine the mechanisms associated with the immunotoxicity of lead [McCabe, 1994; McCabe and Lawrence, 1994; Zelikoff and Cohen, 1996]. Although these results have often been inconsistent, two observations usually hold true. First, lead increases susceptibility to intracellular pathogens and second, polyclonal and/or antigen-specific antibody responses are increased. Experimental models to investigate the mechanisms responsible for these effects are currently being established.

Epidemiologic studies also have been conducted in lead-exposed humans with even less consistent findings.

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Immunologic findings that have been associated with lead exposure in humans include decreased serum immunoglobulin levels [Ewers et al., 1982; Coscia et al., 1987; Jaremin, 1990; Ündeğer et al., 1996], decreased absolute number and percentage of CD4+ cells [Fischbein et al., 1993; Ündeğer et al., 1996], increased absolute number and percentage of B cells [Coscia et al., 1987, Fischbein et al., 1996], decreased number of B cells [Jaremin, 1990], decreased absolute number and percentage of NK cells [Sata et al., 1997], and impaired mitogen-induced lymphoblastogenesis [Fischbein et al., 1993]. In contrast, other investigators have found no difference between lead-exposed subjects and controls in serum immunoglobulin levels [Reigart and Graber, 1976; Kimber et al., 1986; Queiroz et al., 1994], absolute number and percentage of CD4+ cells [Coscia et al., 1987; Cohen et al., 1989; Sata et al., 1997], absolute number and percentage of B cells [Ündeğer et al., 1996; Sata et al., 1997], absolute number and percentage of NK cells [Fischbein et al., 1993; Ündeğer et al., 1996], or mitogen-induced lymphoblastogenesis [Kimber et al., 1986; Cohen et al., 1989; Queiroz et al., 1994].

We conducted a cross-sectional study of lead-exposed and unexposed workers using a comprehensive panel of immunologic parameters to evaluate the association between lead exposure and changes in the immune system. In particular, studies were focused on determining whether the number of CD4+ T cells is affected by lead exposure because a marked decrease in the number of CD4+ cells among lead-exposed subjects compared to controls was reported shortly before the current study was initiated [Fischbein et al., 1993].

MATERIALS AND METHODS

Study Population

Lead-exposed workers were recruited from a large secondary lead smelter in the United States. The unexposed comparison group was recruited from a nearby hardware manufacturing company where no lead is processed. The primary product of the hardware manufacturing company is metal door hinges. Employees received letters explaining the study. In the letter, male workers on first and second shift who had been hired at least 6 months prior to the study were invited to participate. Women were not invited because few women were employed in lead-exposed areas of the secondary lead smelter. Workers taking medications that suppress the immune system (e.g., prednisone), or having serious illnesses of the immune system (e.g., leukemia or AIDS) were asked not to participate. Volunteers were scheduled for appointments until 152 exposed and 86 unexposed workers were recruited for the study. Additional employees were not enrolled for the study due to laboratory resources and constraints. Workers identified during the study to have exposures or conditions that potentially have a significant effect on immune function were excluded from the analysis. This determination was made blind to exposure status. The study protocol was approved by the Human Subjects Review Board of NIOSH, and informed consent was obtained from all workers who participated.

Exposure Characterization

The primary exposure at the secondary lead smelter was lead. Air concentrations of other elements such as arsenic were negligible according to company monitoring data. The comparison group was limited to workers employed in areas of the hardware manufacturing company where exposures to compounds that might affect the immune system were negligible based on a walk-around survey and inspection of the material safety data sheets on process materials. Air sampling for metals and oil mist was conducted at the comparison facility, and concentrations were neglible. Since both facilities consumed considerable amounts of electricity, a walk-through survey of electromagnetic field magnitudes in the extremely low frequency range (ELF) was performed in both plants using a hand-held EMDEX-II field survey monitor (Enertech Consultants, Campbell, CA) using a sampling strategy described elsewhere [Methner, 1996]. The geometric mean ELF magnitudes for the secondary lead smelter and comparison plant were 1.32 and 1.47 milligaus, respectively.

Overview

A questionnaire was administered in person to obtain information on personal demographic data, medical history, medication use, smoking and drinking habits, and work history. Blood from exposed and unexposed workers was collected between 1:00 - 4:30 pm during a 2-week period. Blood was obtained for blood lead and zinc protoporphyrin (ZPP) levels, flow cytometry analysis, hematology, lymphoblastogenesis, natural killer (NK) cell activity, serum immunoglobulin (Ig) G, M, and A concentrations, and serum chemistry analyses. Nonstimulated whole saliva specimens were collected and quantitated for secretory IgA. Blind duplicate specimens were obtained for each test for approximately 10% of the study participants. The results of NK activity are not presented because the correlation between results obtained from blind duplicate specimens was poor ($r=0.15$).

Sample Collection

Blood was withdrawn by antecubital venipuncture into separate tubes containing ACD (acid, citrate, dextrose) for flow cytometry studies, EDTA (ethylenediamine tetraacetic

acid) for hemocytometry, and heparin for lymphoblastogenesis and natural killer cell (NK) assays. Blood was collected in trace element tubes for blood lead analysis and serum separator tubes for the isolation of sera. The serum was separated on site by centrifugation at 1,000 x g for 10 minutes for immunolobulin and other serum chemistry analyses. All blood for immunologic tests was shipped from the study site to the NIOSH laboratory in Cincinnati in insulated boxes that contained recording thermometers to document temperature transients that may have occurred during transport (mean minimum transport temperature was 21.7+/-1.9°C; mean maximum transport temperature was 25.3+/-2.0°C; mean maximum minus minimum transport temperature difference was 3.6+/-1.8°C). Tests performed on fresh blood or isolated cells were initiated within 12-14 hr of phlebotomy. All tests were determined by methods consistent with established guidelines for clinical analysis and in compliance with regulations of the Clinical Laboratory Improvement Act (CLIA).

Flow Cytometry

Whole blood samples were incubated with two- and three- color monoclonal antibodies (Coulter Cytometry, Hialeah, FL) and lysed by a whole blood lysis technique (Multi-QPrep, Coulter). The antibodies used were: CD14-FITC (fluorescein isothiocyanate)/CD45-RD1 (phycoerythrin); CD8-FITC/CD56-RD1; CD4-FITC/CD45RA-RD1; CD4-FITC/CD29-RD1; CD19-FITC/CD2-RD1; and CD8-FITC/CD4-RD1/CD3-ECD (electronically coupled dye, phycoerythrin-Texas red). IgG1-FITC/IgG1-RD1 and IgG1-FITC/IgG1-RD1/IgG2a-ECD were used as isotypic controls. Samples were analyzed on a flow cytometer with a carousel loader (EPICS XL with MCL, Coulter). A tube containing CD45/CD14 was run to obtain a $CD45^{bright}/CD14^{-}$ lymphocyte gate. This gate was held constant for all other tubes in the panel. Resultant data were analyzed with software supplied by the instrument's manufacturer (Coulter). Gated mononuclear cells (3,500) were analyzed for each tube in the panel sample. A 1.0% negative isotype control integration cursor was dynamically set by the software for each tube in the panel, which was then held for all tubes in a specific subject's sample having the same isotype specificity [Biagini et al., 1995; Johanning et al., 1996; CDC, 1997]. Total lymphocyte-subset counts were calculated by multiplying the percent positive by the total number of lymphocytes, measured as outlined below. Blood samples with a lymphocyte purity less than 85% and blood samples in which the sum of the percentage of T (CD3+), B (CD19+), and NK (CD56+) cells was less than 85% or greater than 115% were not included in the analysis of cell markers.

Hematology

Complete blood counts with differentials and reticulocyte counts were performed with a laser hemocytometer (MAXM, Coulter), using reagents and standardization as per the manufacturer's directions [Johanning, et al., 1996].

Clinical Chemistry

Serum levels of C3 (complement component 3), C-reactive protein levels, total levels of IgG, IgM, and IgA, blood lead, ZPP, and salivary IgA were measured either on a centifugal clinical chemistry analyzer (COBAS FARA II, Roche, Nutley, NJ, used per manufacturer's directions), or performed by commercial laboratories (SmithKline Beecham, St. Louis, MO, or MetPath, Teterboro, NJ). Salivary total proteins were measured using a commercial kit (BCA, Pierce, Rockford IL). Antinuclear antibodies were evaluated using a commercial ELISA method (Kallestad ANA Microplate EIA, Sanofi Diagnostics Pasteur, Chaska, MN). Blood lead analyses were performed by a commercial laboratory (SmithKline Beecham, St. Louis, MO, OSHA certified).

Lymphoblastogenesis Assays

Peripheral blood mononuclear cells (PBMC) were isolated for lymphocyte proliferation assays by means of a polysucrose/sodium diatrizoate medium (Histopaque, Sigma Chemical, St. Louis, MO) and Accuspin tubes (Sigma). The cells were resuspended at a concentration of 1×10^6 viable cells per mL (by trypan blue exclusion) in complete assay medium (CM; RPMI 1640 containing 100 units/mL penicillin, 100 µg/mL streptomycin and 2 mM L-glutamine [GIBCO, Grand Island, NY] with 10% FBS. One-hundred µL aliquots of the cell suspensions were dispensed in triplicate into wells of 96-well plates to yield 1×10^5 cells/well. Tetanus toxoid (Wyeth Labs, Philadelphia, PA) was diluted in CM and added at final concentrations of 5, 25, or 50 µg/mL. The first three wells in each row received media in place of antigen and served as a nonstimulated background control. The plates were incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 120 hr. Proliferation was assayed by use of a commercially available cell proliferation/cytotoxicity assay (CellTiter96, Promega, Madison, WI). Briefly, the wells were pulsed for the last 4 hours of incubation with 15 µL of a tetrazolium dye solution to allow viable cells to convert the tetrazolium salt into formazan, which was solubilized and read at a 630 nm absorbance (Dynatech, Alexandria, VA). Stimulation indices (SI) were calculated for the mean of triplicate data for each antigen concentration and vehicle control by the following formula: SI = (OD_{630} with antigen/OD_{630}without antigen).

Statistical Analysis

Baseline characteristics between participants and nonparticipants were compared with t-tests for normally distributed continuous variables, Wilcoxon rank sum tests for skewed continuous variables, and Chi-square tests for categorical variables. Multivariate regression was used to

evaluate the relationship between lead exposure and continuous outcome variables. Logistic regression was used for the dichotomous outcome variables (ANA and C-reactive protein). Variables including age, race, smoking habits, alcohol consumption, marijuana use, whether the subject worked first or second shifts, exposure to 5 or more hours of direct sunlight during the last week, cold or flu symptoms in the last week, hayfever or allergy symptoms in the last week, and hours of sleep the evening prior to the tests were considered candidate covariates. A common set of covariates for all models was selected by evaluating the correlation between these candidate covariates and the dependent variables. The covariates for all final models were age, race, whether the subject worked first or second shifts, and current smoking status. No other covariates were included in the models other than the exposure variable. Although there were numerous outcomes, no formal correction for multiple comparisons was made. Outcome variables were natural log transformed due to their skewed nature. PC-SAS software was used for all statistical procedures [SAS, 1989].

A dichotomous variable indicating whether the subject was occupationally exposed to lead was the initial exposure variable included in the models. These models were used to obtain adjusted geometric mean values in the lead-exposed and unexposed groups for each continuous outcome variable. Unadjusted arithmetic mean values in the lead-exposed and unexposed groups are also presented for comparison with other studies where multivariate analyses were not performed. However, conclusions are based on the results of the multivariate analyses. Among the lead-exposed subjects, multivariate analyses were also conducted in which the dichotomous exposure variable was replaced by a variable for current BLL, current ZPP, or an estimate of cumulative lead exposure. Cumulative lead exposure (in µg/dL days) was estimated for the lead-exposed participants from historical blood lead monitoring results as follows:

$$\int_0^T B(t)dt = \sum_{i=1}^{n-1} \frac{1}{2}(B_i + B_{i+1})\Delta t$$

where B_i and B_{i+1} are the ith and $(i+1)^{th}$ blood lead measurements (in µg/dL), respectively, Δt is the time interval (in days) between measurements, and n is the total number of blood lead measurements. Blood lead monitoring results were available for lead-exposed participants for a median of 4.8 years (range: 0.5 - 18.4 years). Time-integrated blood lead concentration has been reported to correlate with measurements of bone lead, which is considered a measure of cumulative lead exposure [Somervaille et al., 1988; Erkkila et al., 1992].

RESULTS

Seven of the 152 lead-exposed volunteers and 2 of the 86 unexposed volunteers were ineligible, resulting in a final study group of 145 exposed and 84 unexposed workers. The 145 lead-exposed men represented 63% of the eligible population of approximately 230. Participants and nonparticipants at the secondary lead smelter were similar in mean age (32.9 years vs. 34.7 years), but the time since hire for participants was shorter, on average, than for nonparticipants (5.3 vs. 7.7 years, $P=0.009$, Wilcoxon rank sum test). The 84 unexposed men represented 59% of the eligible population of approximately 142 men at the comparison plant. Participants and nonparticipants at the comparison plant were similar in terms of mean age (30.2 vs. 30.3 years) and mean duration of employment (4.3 vs. 4.2 years), but participants were more likely to be white than nonparticipants (62 vs. 43%, $P=0.03$, Chi-square test). Among participants, lead-exposed subjects were slightly older, on average, than unexposed subjects (32.9 vs. 30.1 years, respectively) and were more likely to be nonwhite (60 vs. 38.1%, Table I). All but one of the 119 nonwhite participants were black.

TABLE I. Demographic Information for Workers Exposed to Lead in a Secondary Lead Smelter and Unexposed Workers, 1994

	Exposed Group (N = 145)	Unexposed Group (N = 84)
Age (yrs)	32.9 (8.6)	30.1 (9.3)
Duration of Employment (yrs)	5.3 (4.8)	4.3 (4.3)
Current smoker	49 (33.8%)	36 (42.9%)
Race		
White	58 (40%)	52 (61.9%)
Nonwhite	87 (60%)	32 (38.1%)

Mean (SD) or Number (%).

The median BLL was 39 µg/dL (range: 15-55 µg/dL) among the exposed and <2 µg/dL (range: < 2-12 µg/dL) among the unexposed group. The median ZPP was 48 (range: 2-424 µg/dL) among the exposed and 17.5 µg/dL (range: 1-59 µg/dL) among the unexposed group.

Nine (six exposed and three unexposed) subjects with recent antibiotic, narcotic, corticosteroid, or nonsteroidal anti-inflammatory agent use and eight (seven exposed and one unexposed) with possible or known diabetes mellitus or other illnesses were not included in the analyses. Another 15 (11 exposed and four unexposed) subjects were not included in the analyses of lymphocyte subsets because the sum of their percentage of T (CD3+), B (CD19+), and NK (CD56+) cells was less than 85%.

The arithmetic mean serum immunoglobulins, salivary IgA/protein ratio, C3, leukocyte differential, lymphocyte subsets, and tetanus toxoid-induced lymphoblastogenesis among lead-exposed and unexposed workers are presented in Table II. After adjustment for age, race, work shift, and current smoking status, the geometric mean percentage of monocytes among lead-exposed workers was slightly lower than the geometric mean among unexposed workers (7.8 vs. 8.5%; Table III). There was no other difference, on average,

TABLE II. Arithmetic Mean Results of a Panel of Immunological Tests among Lead-Exposed and Unexposed Workers

Outcome	Exposed	Unexposed
Serum IgA (mg/dL)	223 (92)	196 (93)
Serum IgG (mg/dL)	1177 (304)	1078 (284)
Serum IgM (mg/dL)	121.9 (71.3)	102.6 (46.5)
Salivary IgA/protein	0.77 (0.35)	0.68 (0.30)
Complement C3 (mg/dL)	127 (21)	124 (27)
White Blood Cells ($\times 10^3/\mu$L)	7.4 (2.1)	7.3 (2.0)
Neutrophils ($\times 10^3$ cells/μL)	4.10 (1.64)	3.95 (1.31)
Neutrophils (%)	54.1 (8.4)	53.4 (7.6)
Lymphocytes ($\times 10^3$ cells/μL)	2.47 (0.58)	2.50 (0.84)
Lymphocytes (%)	34.6 (7.6)	34.6 (6.7)
Monocytes ($\times 10^3$ cells/μL)	0.59 (0.19)	0.61 (0.61)
Monocytes (%)	8.1 (2.2)	8.7 (2.1)
Eosinophils ($\times 10^3$ cells/μL)	0.21 (0.17)	0.22 (0.15)
Eosinophils (%)	2.9 (2.0)	3.0 (2.0)
Basophils ($\times 10^3$ cells/μL)	0.01 (0.03)	0.01 (0.03)
Basophils (%)	0.26 (0.33)	0.25 (0.33)
CD56+ (cells/μL)	111 (83)	109 (70)
CD56+ (%)	4.6 (3.3)	4.3 (2.3)
CD19+ (cells/μL)	358 (173)	425 (314)
CD19+ (%)	14.0 (5.2)	16.1 (6.7)
CD3+ (cells/μL)	1892 (480)	1838 (594)
CD3+ (%)	75.6 (7.3)	73.0 (7.7)
CD4+ (cells/μL)	1143 (319)	1132 (435)
CD4+ (%)	45.8 (8.1)	44.5 (7.5)
CD8+ (cells/μL)	606 (228)	600 (243)
CD8+ (%)	24 (6.5)	24.0 (6.2)
CD4+:CD8+ ratio	2.1 (0.9)	2.0 (0.8)
CD45RA+ (cells/μL)	1139 (365)	1196 (519)
CD45RA+ (%)	45.3 (9.6)	46.9 (7.0)
CD4+/CD45RA+ (cells/μL)	475 (224)	498 (287)
CD4+/CD45RA+ (%)	19.0 (8.1)	19.4 (7.2)
CD29+ (cells/μL)	739 (299)	704 (311)
CD29+ (%)	29.3 (8.9)	27.9 (8.3)
CD4+/CD29+ (cells/μL)	642 (228)	601 (292)
CD4+/CD29+ (%)	25.6 (6.9)	23.7 (7.9)
CD4+/CD8+ (cells/μL)	40 (49)	64 (84)
CD4+/CD8+ (%)	1.6 (2.1)	2.4 (2.3)
CD8+/CD56+ (cells/μL)	72.0 (65.7)	100 (98)
CD8+/CD56+ (%)	2.9 (2.3)	3.8 (3.8)
Lymphocyte activation with 5 μg/mL tetanus toxoid (stimulation indices)	118 (10)	118 (8)
Lymphocyte activation with 25 μg/mL tetanus toxoid (stimulation indices)	116 (28)	117 (37)
Lymphocyte activation with 50 μg/mL tetanus toxoid (stimulation indices)	236 (82)	221 (80)

Mean (SD).
Some participants did not have results for all tests. The number of exposed and unexposed participants ranged from 111 to 128 and from 71 to 79, respectively.

TABLE III. Adjusted Geometric Mean Results of a Panel of Immunological Tests among Lead-Exposed and Unexposed Workers*

Outcome	R^2 for model	Adjusted Geometric Mean: Exposed	Adjusted Geometric Mean: Unexposed	*P*-value
Serum IgA (mg/dL)	11.1	202	180	0.07
Serum IgG (mg/dL)	31.0	1110	1090	0.62
Serum IgM (mg/dL)	4.7	106.2	94.5	0.11
Salivary IgA/protein	1.5	0.68	0.59	0.16
Complement C3 (mg/dL)[b]	11.3	125	123	0.57
White Blood Cells ($\times 10^3/\mu$L)	19.5	7.2	6.9	0.29
Neutrophils ($\times 10^3$ cells/μL)	21.0	3.88	3.60	0.14
Neutrophils (%)	12.6	54.0	52.0	0.11
Lymphocytes ($\times 10^3$ cells/μL)[b]	11.9	2.41	2.42	0.84
Lymphocytes (%)	13.1	33.2	34.9	0.12
Monocytes ($\times 10^3$ cells/μL)	8.8	0.56	0.59	0.27
Monocytes (%)	5.1	7.8	8.5	0.03[a]
Eosinophils ($\times 10^3$ cells/μL)	6.6	0.17	0.17	0.80
Eosinophils (%)	1.2	2.4	2.5	0.89
Basophils ($\times 10^3$ cells/μL)	2.3	0.01	0.01	0.81
Basophils (%)	2.3	0.2	0.2	0.97
CD56+ (%)	1.5	3.5	3.6	0.84
CD19+ (%)	5.1	13.2	14.6	0.13
CD3+ (%)	5.5	74.9	73.1	0.14
CD4+ (%)	10.6	44.6	44.7	0.92
CD8+ (%)	8.3	23.3	23.0	0.72
CD4+:CD8+ ratio	12.0	1.9	1.9	0.85
CD45RA+ (%)	8.6	44.1	45.6	0.43
CD4+/CD45RA+ (%)	5.6	17.2	17.9	0.56
CD29+ (%)	1.6	27.7	25.8	0.29
CD4+/CD29+ (%)	17.1	24.0	22.5	0.29
CD4+/CD8+ (%)	5.3	0.9	1.5	0.003[a]
CD8+/CD56+ (%)	11.0	2.2	2.8	0.04[a]
Lymphocyte activation with 5 μg/mL tetanus toxoid (stimulation indices)	1.1	118	117	0.60
Lymphocyte activation with 25 μg/mL tetanus toxoid (stimulation indices)	1.3	114	112	0.71
Lymphocyte activation with 50 μg/mL tetanus toxoid (stimulation indices)	3.8	221	206	0.23

*All models had a variable for lead exposed vs unexposed. All models also included age, race, current smoking status, and work shift and no other variables besides exposure. All outcome variables reported here were natural log transformed prior to analysis, then untransformed for reported results. A constant was added to the percentage of basophils prior to transformation. Some participants did not have results for all tests. The number of exposed and unexposed participants ranged from 113 to 128 and from 71 to 79, respectively.
[a]*P*-value < 0.05.
[b]A significant interaction between the dichotomous exposure variable and age was included in the model.

in the leukocyte differential between lead-exposed and unexposed workers, but there was significant interaction between age and the dichotomous lead exposure variable in the model for the number of lymphocytes ($P=0.007$). The number of lymphocytes was lower among older exposed workers than older unexposed workers and higher among younger exposed workers than younger unexposed workers. The interaction seems to be due to an increased number of lymphocytes with increasing age in the unexposed population. The number of lymphocytes did not vary significantly with age in the exposed population. Since the number of lymphocytes expressing specific cell markers (such as CD4+) is derived from the total number of lymphocytes and the percentage of cells expressing the cell markers, these analytic models also showed an interaction for age and exposure, although the interaction was not always statistically significant at the 0.05 level (results not shown). For example, significant interaction between age and the dichotomous lead exposure variable was found for the absolute number of CD4+ T cells ($P=0.05$), but not for the percentage of CD4+ T cells ($P=0.50$).

After adjustment for age, race, work shift, and current smoking status, the percentage of CD4+/CD8+ cells and the percentage of CD8+/CD56+ cells were slightly lower, on average, among the exposed workers (0.9 vs. 1.5% and 2.2 vs. 2.8%, respectively; Table III). There was no difference, on average, in the percentages of other lymphocyte subsets, serum immunoglobulin levels, salivary IgA/protein ratio, C3 levels, or lymphocyte activation with tetanus toxoid between exposed and unexposed workers.

Table IV shows the results of analyses for the lead-exposed group by current BLL, ZPP, and estimated cumulative lead exposure. Increasing BLLs were associated with an increase in the absolute number and percentage of CD19+ cells. BLLs were not significantly associated with the number or percentage of CD4+ T cells, other lymphocyte subsets, serum immunoglobulin levels, salivary IgA/protein ratio, C3, leukocyte differential, or lymphocyte activation with tetanus toxoid. One outlier was excluded in the analyses of immunologic outcomes by ZPP levels. Increasing ZPP levels were significantly associated with a decrease in the percentage of monocytes. When the outlier was included in the analysis, increasing ZPP levels were significantly associated with a decrease in the percentage of monocytes and an increase in the number of CD3+ cells, CD4+ T cells, CD8+ T cells, and CD45RA+/CD4+ cells. The estimate of cumulative lead exposure was associated with a decrease in serum IgG and with an increase in the number and percentage of CD4+/CD45RA+ cells. Estimated cumulative lead exposure was not associated with other serum immunoglobulins, CD4+ T cells, other lymphocyte subsets, salivary IgA/protein ratio, C3, leukocyte differential, or tetanus toxoid induced lymphoblastogenesis.

Logistic regression results (not shown) for dichotomous outcome variables indicated no significant differences between exposed and unexposed workers for a C-reactive protein greater than 8 units or for a positive ANA. Furthermore, there was no relationship between these variables and current BLLs, ZPP levels, or the estimate of cumulative lead exposure.

DISCUSSION

Fischbein and colleagues [1993] reported that firearms instructors with BLLs ≥25 μg/dL (mean BLL=31.4 μg/dL) had a 48% reduction and firearms instructors with a BLL < 25 μg/dL had a 29% reduction in the number of CD4+ cells compared to unexposed controls. The firearms instructors also had a reduction in the percentage of CD4+ cells, a reduction in the number and percentage of T (CD3+) cells, impaired T-cell function as determined by proliferative response to mitogens, and decreased mixed lymphocyte culture responses. Ündeğer and colleagues [1996] also reported a reduction in the absolute number and percentage of CD4+ cells among a group of lead-exposed workers with a mean BLL of 74.8 μg/dL compared to controls. In contrast, other investigators have observed no difference in the absolute number and percentage of CD4+ cells between lead-exposed subjects and controls [Cohen et al., 1987; Coscia et al., 1987; Sata et al., 1997].

In this study, we found no association between lead exposure and the percentage of CD4+ T cells. Although we found a significant interaction between age and lead exposure status for the absolute number of CD4+ T cells, this finding may be related to an increase in lymphocyte count with age in the unexposed population rather than a change in the effect of lead with age. However, data from National Health and Nutrition Examination Surveys indicate that lymphocyte counts do not increase with age among adult males [van Assendelft, 1985]. This suggests that the difference in the number of CD4+ T cells observed between exposed and unexposed workers may not be related to lead exposure but to another factor in the unexposed group. In exposure-response analyses among lead-exposed workers, we found no evidence of a significant relationship between the number and percentage of CD4+ cells and estimates of exposure including current blood lead, ZPP, or estimated cumulative lead exposure. These findings do not support the previous findings that lead exposure is associated with a marked reduction in the number and percentage of CD4+ cells [Fischbein et al., 1993; Ündeğer et al., 1996].

There are some methodologic differences between our study and the studies by Fischbein et al. [1993] and Ündeğer et al. [1996] that may account for these contrasting findings. Circadian variations of CD4+ T cell counts have been reported [Bertouch et al., 1983; Ritchie et al., 1983]. In the present study, most blood was collected between 1:00 pm

TABLE IV. Immunological Outcomes as a Function of Current Blood Lead, Zinc Protoporphyrin, and Cumulative Lead Exposure among Lead-Exposed Workers*

	Blood Lead Level		Zinc Protoporphyrin[b]		Cumulative Lead Exposure	
Ln (Outcome)	**Direction**	***P***	**Direction**	***P***	**Direction**	***P***
Serum IgA (mg/dL)	+	0.82	+	0.21	−	0.33
Serum IgG (mg/dL)	−	0.72	−	0.83	−	0.03[a]
Serum IgM (mg/dL)	−	0.91	−	0.13	−	0.10
Salivary IgA/protein	−	0.95	+	0.89	+	0.46
Complement C3 (mg/dL)	+	0.99	+	0.84	−	0.71
White blood cells ($\times 10^3$ cells/μL)	−	0.94	+	0.73	+	0.14
Neutrophils ($\times 10^3$ cells/μL)	−	0.58	+	0.54	+	0.17
Neutrophils (%)	−	0.26	+	0.42	+	0.46
Lymphocytes ($\times 10^3$ cells/μL)	+	0.40	+	0.69	+	0.42
Lymphocytes (%)	+	0.30	+	0.98	−	0.42
Monocytes ($\times 10^3$ cells/μL)	−	0.71	−	0.16	+	0.35
Monocytes (%)	−	0.70	−	0.04[a]	−	0.76
Eosinophils ($\times 10^3$ cells/μL)	−	0.95	−	0.32	+	0.62
Eosinophils (%)	−	0.97	−	0.24	−	0.96
Basophils ($\times 10^3$ cells/μL)	+	0.96	+	0.48	+	0.99
Basophils (%)	+	0.94	+	0.52	−	0.70
CD56+ (cells/μL)	−	0.63	+	0.23	+	0.60
CD56+ (%)	−	0.43	+	0.27	+	0.96
CD19+ (cells/μL)	+	0.006[a]	+	0.13	−	0.38
CD19+ (%)	+	0.01[a]	+	0.45	−	0.17
CD3+ (cells/μL)	+	0.33	+	0.37	+	0.21
CD3+ (%)	−	0.75	−	0.52	+	0.51
CD4+ (cells/μL)	+	0.38	+	0.26	+	0.12
CD4+ (%)	−	0.76	−	0.71	+	0.21
CD8+ (cells/μL)	+	0.41	+	0.32	+	0.51
CD8+ (%)	+	0.84	+	0.40	−	0.79
CD4+:CD8+ ratio	−	0.86	−	0.87	+	0.65
CD45RA+ (cells/μL)	+	0.30	+	0.61	+	0.77
CD45RA+ (%)	+	0.63	−	0.92	−	0.64
CD4+/CD45RA+ (cells/μL)	+	0.45	+	0.41	+	0.003[a]
CD4+/CD45RA+ (%)	+	0.93	−	0.54	+	0.002[a]
CD29+ (cells/μL)	−	0.33	−	0.25	−	0.86
CD29+ (%)	−	0.10	−	0.14	−	0.36
CD4+/CD29+ (cells/μL)	+	0.62	+	0.65	+	0.56
CD4+/CD29+ (%)	−	0.69	−	0.72	−	0.76
CD4+/CD8+ (cells/μL)	+	0.49	+	0.30	−	0.82
CD4+/CD8+ (%)	+	0.64	+	0.45	−	0.66
CD8+/CD56+ (cells/μL)	−	0.18	−	0.41	+	0.43
CD8+/CD56+ (%)	−	0.08	−	0.31	+	0.67
Lymphocyte activation with 5 μg/ml tetanus toxoid (stimulation indices)	+	0.57	−	0.96	−	0.90
Lymphocyte activation with 25 μg/ml tetanus toxoid (stimulation indices)	−	0.99	−	0.96	+	0.16
Lymphocyte activation with 50 μg/ml tetanus toxoid (stimulation indices)	−	0.62	−	0.38	−	0.17

*All models included age, race, current smoking status, and work shift and no other variables besides exposure (current blood level, zinc protoporphyrin, or estimated cumulative lead exposure).
The outcome variable in all models was natural log transformed. A constant was added to the percentage of basophils prior to transformation.
Cumulative lead exposure was estimated from historical blood lead data.
Some participants did not have results for all tests. The number of exposed participants ranged from 109 to 128.
[a]*P*-value < 0.05.
[b]One outlier was excluded in the analyses of immunological outcomes as a function of zinc protoporphyrin.

and 4:30 pm, workers on third shift were excluded, and a variable was included in the analyses to adjust for work shift (first/second) to minimize the effect of this diurnal variation in CD4+ T-cell counts on the study findings. We also excluded blood samples with a lymphocyte purity less than 85% and blood samples in which the sum of the percentage of T (CD3+), B (CD19+), and NK (CD56+) cells was less than 85% from the analysis of the CD4+ T cell count. The reports by Fischbein et al. [1993] and Ündeğer et al. [1996] do not describe using these restrictions. In fact, the sum of the mean percentage of T (CD3+), B (CD20+), and NK (CD16+) cells in the study by Fischbein and colleagues [1993] was 95.9% among controls, 82.9% among firearms instructors with a BLL $<$ 25 µg/dL, and only 72.6% among firearms instructors with a BLL $\geq$ 25 µg/dL. According to guidelines developed by the Centers for Disease Control and Prevention, these values should be 90-110% [CDC, 1997]; values less than this may indicate a quality control problem in immunophenotyping due to high nonlymphocyte contamination of the lymphocyte gate. Exposure differences may explain the discrepant findings between the present study and those by Ündeğer et al. [1996]. The mean BLL among the 25 lead-exposed subjects in the study by Ündeğer et al. [1996] was higher than the maximum current BLL observed in the present study (74.8 µg/dL vs. 55 µg/dL, respectively).

Fischbein and colleagues [1993] had observed decreased lymphocyte responsiveness to phytohemagglutinin and pokeweed mitogen. However, we found no association between lead exposure and lymphocyte proliferation with tetanus toxoid. This is consistent with other investigators who found no association between lead exposure and blast transformation of lymphocytes with phytohemagglutinin or Conconavalin A [Kimber et al., 1986; Cohen et al., 1989; Queiroz et al., 1994].

Whereas Fischbein et al. [1993] and Coscia et al. [1987] reported an increase in the percentage and number of B (CD20+) cells among lead-exposed workers compared to unexposed controls, Ündeğer et al. [1996] and Sata et al. [1997] found no association and Jaremin [1990] reported a reduction in the number of B cells among exposed workers with 'lead poisoning traits.' Findings of this study were similar to Fischbein and Coscia in that the percentage and number of B (CD19+) cells were positively associated with current BLLs among lead-exposed workers. However, there was no difference in the percentage of B cells, on average, between lead-exposed and unexposed workers.

The current study found no difference in salivary IgA or serum immunoglobulin levels between exposed and unexposed workers. These findings are similar to three other studies [Reigart and Graber, 1976; Kimber et al., 1986; Quieroz et al., 1994], but in contrast to several other previous studies that found lower levels of serum and salivary IgA [Ewers et al., 1982], serum IgM [Ewers et al., 1982; Coscia et al., 1987; Jaremin, 1990; Ündeğer et al., 1996], and serum IgG [Ewers et al., 1982; Jaremin, 1990; Ündeğer et al., 1996] among lead-exposed subjects compared to controls. Only three studies in addition to the current one have examined the relationship between individual BLLs and serum immunoglobulin levels [Ewers et al., 1982; Coscia et al., 1987; Ündeğer et al., 1996]. None of the studies, including the current one, found a correlation between BLLs and serum IgM. Serum IgA levels were not correlated with BLLs in this study or in two of the other studies [Coscia et al., 1987; Ündeğer et al., 1996]. However, decreasing serum IgG levels were correlated with increasing BLLs in two of the studies [Ewers et al., 1982; Ündeğer et al., 1996]. IgG levels were not correlated with BLLs in this study, but decreasing IgG levels were associated with increasing cumulative lead exposure. The difference in BLLs may account for some of the differences in findings between studies. The mean BLLs in studies with no difference in serum immunoglobulins between lead-exposed subjects and controls were, in general, lower than in studies where lead-exposed workers had lower levels of serum immunoglobulins.

We found that, on average, lead-exposed workers had a significantly lower percentage of monocytes than unexposed workers, although the magnitude of the difference was small. In exposure-response analyses, the percentage of monocytes was found to decrease with increasing ZPP among the exposed subjects. Previous epidemiologic studies on the immunologic effects of occupational lead exposure have not reported results for the percentage or number of monocytes. Exposed workers also had fewer CD4+/CD8+ cells (immature T cells) and CD8+/CD56+ cells, on average, than unexposed workers, but we found no evidence of an exposure-response relationship between these cell types and blood lead, ZPP, or estimated cumulative lead exposure.

Although we found no difference in the percentage of CD4+ T cells or the subpopulations of CD4+ T cells between the exposed and unexposed workers, the percentage of CD4+/CD45RA+ cells (recent thymic emigrants) was positively associated with the estimate of cumulative lead exposure among exposed workers. No other study has evaluated CD4+ T-cell subpopulations among lead-exposed workers.

Methodologic strengths of this study include the selection of a highly exposed lead population, adequate population size, control for potential circadian rhythm by restricting the time of blood draws, and measuring a large number of immunologic parameters. The study has some limitations. Since workers volunteered for the study after receiving a letter inviting them to participate, self-selection bias may have occurred. The study also has the weaknesses inherent to a cross-sectional study design. Another limitation of this and most epidemiologic studies of immunotoxic agents is

that individual markers of immune function were measured rather than integrated measures of immune function such as primary or secondary antibody response or susceptibility to infection. This limitation is particularly important for assessment of the effects of lead since the strongest effects in animal studies are found by measuring response to infection challenges. Given this limitation, this study does not provide evidence for a marked immunotoxic effect of lead at the exposure levels studied. The few statistically significant results that were detected could represent type I errors, associations that occur by chance, given the large number of tests that were done. Although some associations were observed, the magnitude of the differences observed were small. For example, serum IgG levels decreased with increasing cumulative lead exposure, but the magnitude of the association is small. Interpretation of such findings is difficult because there are few quantitative human data on the degree to which such parameters need to be modified in a population before an increased risk of disease can be observed.

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