

Decreases in CD8⁺ T, Naive (CD4⁺CD45RA⁺) T, and B (CD19⁺) Lymphocytes by Exposure to Manganese Fume

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Abstract: To examine the effects of exposure to manganese (Mn) on the cellular and humoral immune system in men, T lymphocyte subpopulations, B (CD19⁺) lymphocytes, natural killer (NK) cells, and serum immunoglobulins (i.e., IgG, IgA and IgM) together with total T (CD3⁺) lymphocytes and total lymphocytes were measured in blood samples from 21 welders mainly exposed to Mn fume with blood Mn (BMn) concentrations of 0.6–2.3 (mean 1.4) $\mu\text{g}/\text{dl}$ and 21 healthy controls working in the same factory (BMn concentrations: 0.7 to 1.7, mean 1.1 $\mu\text{g}/\text{dl}$). The workers engaged in welding for 6 to 36 (mean 17) yr. All the study subjects were divided into 3 equally sized groups (n=14 for each group) according to BMn concentrations. Numbers of CD8⁺ T, total T (CD3⁺), B (CD19⁺), and total lymphocytes were significantly lower in high-BMn group than those in low-BMn group; the numbers of CD8⁺ T lymphocytes were significantly lower in moderate-BMn group compared to low-BMn group. After adjusting for age and smoking, significant inverse correlations between BMn concentrations and CD4⁺CD45RA⁺ T, CD4⁺ T, CD8⁺ T, CD3⁺ T, and total lymphocytes were found. We conclude that T lymphocytes, especially CD8⁺ and CD4⁺CD45RA⁺ T lymphocytes, as well as CD19⁺ B lymphocytes are affected by exposure to Mn fume.

Key words: Manganese, Naive (CD4⁺CD45RA⁺) T lymphocyte, CD8⁺ T lymphocyte, CD19⁺ B lymphocyte, Welders, Occupational exposure, Immunotoxicity, Lymphocyte subpopulation

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Introduction

The effects of occupational exposure to Manganese (Mn) on the human immune system have not been fully elucidated. A decrease in serum immunoglobulin G (IgG) concentrations without changes in IgA and IgM concentrations has been found in 74 male welders with an average air Mn concentration of 0.45 mg/l¹⁾. A significant decrease in the percentage of activated T (CD3+HLA-DR+) lymphocytes without changes in the absolute numbers of total T (CD3+), CD3+HLA-DR+ T, CD4+ T, and CD8+ T lymphocytes and CD57+ natural killer (NK) cells has been reported in workers exposed to ethylene-bis-dithiocarbamate of Mn and zinc (Mancozeb) with an average blood Mn (BMn) concentration of 0.29 µg/dl²⁾. On the other hand, it appears that no study had been reported on the effects of occupational exposure of Mn on CD4+ T lymphocyte subpopulations.

CD4+ T lymphocytes are divided into two main phenotypic subpopulations, i.e., CD4+CD45RO+ (memory) and CD4+CD45RA+ (naive) T lymphocytes subsets³⁾. Evaluation of this subpopulation would provide more precise information on the effects of Mn on T lymphocytes than mere measurement of total CD4+ T lymphocytes.

In the present study, to clarify the effects of welding and exposure to Mn on the cellular and humoral immune system, we measured CD4+ T lymphocyte subpopulations together with CD8+ T lymphocytes, and B (CD19+) lymphocytes, CD56+/CD16+ NK cells, and serum immunoglobulins (IgG, IgA and IgM) in welders mainly exposed to Mn fume.

Subjects and Methods

Subjects

Twenty-one male welders, aged 29 to 56 (mean 43) yr, at 5 factories in Korea were examined. The factories were manufacturing parts of bridge, pulverization machines, air conditioners, textiles or window glasses. At the time of this study, BMn concentrations in all these workers ranged from 0.6 to 2.3 µg/dl with a mean of 1.4 µg/dl, which were significantly higher than those in their controls as shown below (Student's *t* test, *p*<0.01). The welding fume from alloy-steel bars contained 0.5–3.0% of Mn and less than 0.0036% of lead (Pb). The workers engaged in welding for 6 to 36 (mean 17) yr.

The concentration of Mn in the air of workplace was 35.7 mg/m³ at the factory producing bridge parts and 4.8 mg/m³ at the factory producing air conditions; the concentrations at other 3 factories were not available. The threshold limit value by American Conference Governmental Industrial

Hygienist (ACGIH)⁴⁾ Standard was 0.2 mg/m³; the shift time weighted average (TWA) value for Mn concentrations in the air at work was 0.15 mg/m³. Occupational exposure limits for Mn and compounds by the Japan Society for Occupational Health were 0.3 mg/m³⁵⁾.

Control subjects, aged 30 to 57 (mean 43) yr, were 21 healthy male clerks, security guards and drivers at the same factory. BMn concentrations of 21 controls ranged from 0.7 to 1.7 (mean 1.1) µg/dl.

Routine clinical, hematological and biochemical examinations showed no abnormalities in welders and in control subjects. They were instructed to refrain from drugs or alcohol from the day before the study (at least 12 h prior to measurements). At the time of the study, none of the workers or controls had signs or symptoms indicative of infection or had been administered any medicine which could affect immunological analyses. Nature of the procedure in the present study was fully explained to all subjects, and the study was carried out with their informed consent. The subjects were told that they could discontinue to participating the experiment any time they want without sustaining any possible disadvantages. Although the institutional review board for epidemiological study did not exist at the University of Tokyo in 1997, we followed the recommendations for human studies outlined in the Declaration of Helsinki.

Determination of BMn

For determination of BMn, whole blood was collected in heparinized vacutainer tubes (Becton Dickinson, San Jose, California, USA). BMn concentration was measured by Atomic Absorption Spectrophotometer (AAS, Varian Australia PTY LTD, Australia) with graphite furnace by a modified analytical method for graphite tube atomizers.

Measurement of lymphocyte subpopulations

Venous blood samples were taken at 8:00 to 10:00 a.m., for immunological analysis. Ethylenediaminetetraacetic acid (EDTA) was used as an anticoagulant and was utilized for measurement of leukocyte counts and immunofluorescence staining. All samples were transported and handled at room temperature (i.e., 15–20°C).

Immunological analysis was conducted within 12 h after the collection of samples. Measurements of lymphocyte subpopulations immunofluorescence surface marker analysis using the following sets of monoclonal antibodies: anti-2H4 (CD45RA-FITC)/anti-T4 (CD4-PE), anti-T4 (CD4-FITC)/anti-UCHL1 (CD45RO-PE), anti-T3 (CD3-FITC)/anti-B4 (CD19-PE), anti-T3 (CD3-FITC)/anti-3G8+N901/NKH-1 (CD56+/CD16+)-PE, anti-T8 (CD8-FITC)/anti-S6F1

(CD11a-PE) and mouse IgG1-FITC/mouse IgG1-PE (negative control). All antibodies were purchased from Beckman Coulter Inc. (Hialeah, Florida, USA). Lymphocyte subpopulations were determined by flow cytometry analysis (EPICS XL, Beckman Coulter Inc.) according to standard methods⁶⁻⁸⁾. Total lymphocyte counts were measured by the automated cell count analyzer (STKR, Coulter Counter SP-VI, Coulter Electronics, Hialeah, Florida, USA). The number of each lymphocyte subpopulation was calculated by multiplying the percentage of positive cells in each subpopulation category determined by the flow cytometry by total lymphocyte counts.

Immunoglobulin assay

Serum IgG, IgA and IgM concentrations were determined by the radical immunodiffusion technique according to Turbidimetric Immunoassay (TIA; Hitachi automatic analyzer 7150, Tokyo, Japan)⁹⁾. This instrument automatically measures and compensates for the initial absorbance of the reaction mixture within 2.5 s of mixing of the reactants, including the production of individual blanks. Turbidity was measured at 340 nm.

Statistical analysis

All the subjects including controls were subdivided into three equally sized groups according to their BMn concentrations to examine the relationship between BMn concentrations and immune parameters; a high-BMn group (1.3 to 2.3 (mean 1.7) $\mu\text{g}/\text{dl}$, $n=14$), a moderate-BMn group (1.0–1.3 (mean 1.1) $\mu\text{g}/\text{dl}$, $n=14$) and a low-BMn group (0.6 to 1.0 (mean 0.9) $\mu\text{g}/\text{dl}$, $n=14$). Differences in the numbers of all lymphocyte subpopulations and serum immunoglobulin concentrations among high-BMn, moderate-BMn, and low-BMn groups were analyzed by the analysis of covariance with age and cigarettes/day as covariates or analysis of variance. Differences in BMn concentration and the number of cigarettes smoked per day were calculated by analysis of variance. Fisher LSD post-hoc test was used to identify which group differences accounted for the significant p value. Age and smoking-adjusted (i.e., partial) correlation coefficients between BMn and lymphocyte subpopulations or serum immunoglobulins in all subjects were calculated to know dose-effect relationships between BMn concentrations and immune parameters; we also calculated correlation coefficients between exposure period and immune parameters in 21 welders. All data in this study were analyzed by the Statistical Package for Social Sciences, version 13.0 (SPSS Inc., Chicago, USA).

Results

Differences in BMn concentrations, numbers of lymphocyte subpopulations, serum immunoglobulins, and number of cigarettes smoked per day in high, moderate, or low BMn groups are shown in Table 1. Numbers of CD8+ T, total T (CD3+), B (CD19+), and total lymphocytes were significantly lower in high-BMn group compared to low-BMn group; the number of CD8+ T lymphocytes was significantly lower in moderate-BMn group compared to low-BMn group.

Significant inverse correlations were found between BMn concentrations and CD4+CD45RA+ T, CD4+ T, CD8+ T, CD3+ T, and total lymphocytes (partial correlation analysis with the effects of age and cigarettes/day eliminated, $p<0.05$) (Table 2). Neither lymphocyte subpopulations nor immunoglobulins were significantly correlated with duration of Mn exposure in 21 welders.

Discussion

Significant decreases in CD8+ T and CD19+ B lymphocytes were found in high-BMn workers compared to low-BMn workers; lower counts in CD8+ T lymphocytes were also found in moderate-BMn workers compared to low-BMn workers. In addition, numbers of CD4+CD45RA+ T, CD4+ T, CD8+ T, and CD3+ T lymphocytes were inversely correlated with BMn concentrations ($p<0.05$). We conclude that T lymphocyte subpopulations and B lymphocyte are specifically affected in workers chronically exposed to Mn. Decreases in CD4+CD45RA+ and CD8+ T lymphocytes may account for decreases in total T (CD3+) lymphocytes and total lymphocytes.

This is probably the first report that disclosed selective decreases in CD8+ T, CD4+CD45RA+ T, and CD19+ B lymphocytes in welders mainly exposed to Mn. It appears that these lymphocytes are sensitive subclinical markers on the human effects of Mn as it was significantly decreased despite the fact that the differences in BMn concentrations among high-, moderate-, low-BMn groups were not so large.

Colosio *et al.*²⁾ found no changes in the percentages of total CD4+, CD8+ T and total T (CD3+) lymphocytes in workers exposed to Mancozeb with an average blood concentration of 0.29 $\mu\text{g}/\text{dl}$ despite the fact that they found a decrease in the percentage of activated CD3+ T (CD3+HLA-DR+) lymphocytes. However, they found a significant decrease in response to lymphocyte proliferation test to phytohemagglutinin A. A possible explanation is that function of T lymphocytes especially CD4+ and CD8+ T

Table 1. Differences in BMn concentrations (ug/dl), numbers of lymphocyte subpopulations (per mm³ whole blood), serum immunoglobulin concentrations (ug/dl), and number of cigarettes smoked per day according to BMn concentrations in welders and controls combined (mean values with ranges in parentheses).

	High-BMn (n=14)	Moderate-BMn (n=14)	Low-BMn (n=14)
BMn concentration	1.7 (1.3–2.3) ^{a, b}	1.1 (1.0–1.3) ^a	0.9 (0.6–1.0)
T lymphocytes:			
CD4+CD45RA+	316 (176–539)	338 (164–569)	361 (196–511)
CD4+CD45RO+	528 (339–825)	556 (314–902)	614 (436–1151)
Total CD4+	800 (532–1011)	880 (543–1418)	964 (619–1499)
CD8+	477 (324–665) ^d	471 (290–771) ^c	588 (384–913)
Total T (CD3+)	1,115 (853–1426) ^d	1,215 (827–1653)	1,369 (1094–1862)
B (CD19+) lymphocytes	191 (86–379) ^d	254 (100–506)	340 (136–658)
NK (CD56+/CD16+) cells	350 (181–730)	490 (219–851)	385 (182–724)
Total lymphocytes	1,726 (1350–2385) ^{d, e}	2,034 (1311–2562)	2,187 (1596–2850)
Immunoglobulins:*			
IgG	1,271 (850–1590)	1,122 (866–1500)	1,144 (913–1360)
IgA	227 (145–414)	177 (85–344)	213 (100–397)
IgM	113 (55–168)	94 (60–129)	102 (60–186)
Cigarettes smoked per day	7.1 (0–40)	10.0 (0–20)	10.5 (0–20)

^a $p < 0.001$ (analysis of variance with Fisher LSD post-hoc comparison; compared to low-BMn group)

^b $p < 0.01$ (analysis of variance with Fisher LSD post-hoc comparison; compared to moderate-BMn group)

^c $p < 0.05$, ^d $p < 0.01$ (analysis of covariance with age and smoking as covariates with Fisher LSD *post-hoc* comparison; compared to low-BMn group)

^e $p < 0.05$ (analysis of covariance with age and smoking as covariates with Fisher LSD post-hoc comparison; compared to moderate-BMn group)

* Geometric means.

Table 2. Relationships between BMn concentrations (ug/dl) and numbers of lymphocyte subpopulations (per mm³ whole blood) and serum immunoglobulins (ug/dl) (n=42)

	BMn concentrations
T lymphocytes:	
CD4+CD45RA+	-.313 ^a
CD4+CD45RO+	-.227
Total CD4+	-.327 ^a
CD8+	-.418 ^b
Total T (CD3+)	-.480 ^b
B (CD19+) lymphocytes	-.300
NK (CD56+/CD16+) cells	-.175
Total lymphocytes	-.557 ^c
Immunoglobulins:*	
IgG	.163
IgA	-.031
IgM	.008

^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$ (Partial correlations controlling for age and numbers of cigarettes smoked per day).

* Geometric means.

lymphocytes are affected by chronic low-dose exposure to Mn while higher dose may affect not only function but quantity of lymphocytes. Another explanation is that the study by Colosio *et al.*²⁾ measured only percentages of lymphocyte subpopulations but not absolute counts which is more sensitive to chemical agents^{8, 11, 12, 16–20}.

Strong inverse correlations between BMn concentrations and CD4+CD45RA+ T, CD4+ T, CD8+ T, and CD3+ T lymphocytes were found in the current study. Since CD4+CD45RA+ T lymphocytes were decreased by exposure to Mn, naïve (CD45RA+) population of lymphocytes (CD8+CD45RO+ and CD19+CD45RA+) may be reduced. This may account for decreases in CD4+ T, CD8+ T, and CD19+ B lymphocytes. Further investigation is necessary to confirm this hypothesis.

No significant decreases in serum immunoglobulins were found in the present study. In contrast, Bonshnakova *et al.*¹⁾ found a significant decrease in serum IgG in welders exposed to Mn. Serum IgA and IgM were not affected by Mn exposure in both the present and Bonshnakova's study¹⁾. Also, there

was no significant relationship between Igs and lymphocyte subpopulations suggesting that the cellular and humoral immune system is independently affected by exposure to Mn. Thus, it may be considered that exposure to Mn may have no effect or a very weak effect on serum IgG.

In this study, subjects were also exposed to a low level of lead. Exposure to lead has been reported to have a decreasing effect on immune cells such as CD4⁺ T lymphocytes¹⁰⁾, CD3⁺CD45RO⁺ T lymphocytes¹¹⁾, and CD16⁺ NK cells¹²⁾, as well as serum IgG, A and M in a relatively high level as 20 µg/dl in the peripheral blood¹³⁾. However, the welders in this study were exposed to the welding fume from alloy-steel bars containing less than 0.0036% of lead suggesting a very low level exposure to lead, which may be negligible.

Recently, immunological effects of occupational and environmental chemicals have been reported in a series of studies. Fischebein *et al.*¹⁰⁾ reported a decrease in the number of CD4⁺ T lymphocytes in workers exposed to lead. Denkhaus *et al.*¹⁴⁾ and McConnachie *et al.*¹⁵⁾ showed a decrease in the percentage of CD4⁺ T lymphocytes in workers exposed to mixtures of organic solvents and pentachlorophenol, respectively. In our previous reports, we found a significant decrease in the number of CD4⁺ T lymphocytes in workers exposed to aromatic amines¹⁶⁾ and chromates¹⁷⁾. The decrease in the number of CD4⁺CD45RA⁺ T lymphocytes in workers exposed to benzidine and beta-naphthylamine¹⁸⁾, chromates¹⁹⁾ and mixed organic solvents²⁰⁾ were also found in our previous studies. Furthermore, a study from our group showed a significant reduction in the number of memory T (CD3⁺CD45RO⁺) lymphocytes in 71 lead-exposed male workers¹¹⁾. Thus, CD4⁺ T lymphocyte subpopulations may be affected differently by the types of occupational and environmental chemicals exposed. Additional studies are required to clarify the mechanism underlying the different effects of these substances.

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