Toxicol. Res.
Vol. 29, No. 2, pp. 115-120 (2013)
Open Access http://dx.doi.org/10.5487/TR.2013.29.2.115
pISSN: 1976-8257 eISSN: 2234-2753

Toxicological Research
Official Journal of
Korean Society of Toxicology
Available online at http://www.toxmut.or.kr

Effects of Beryllium on Human Serum Immunoglobulin and Lymphocyte Subpopulation

Ki-Woong Kim[1], DaeSeong Kim[2], Yong Lim Won[1] and Seong-Kyu Kang[3]

[1]Occupational Safety and Health Research Institute, KOSHA, Incheon, Korea
[2]Korea University Ansan Hospital, Ansan, Korea
[3]Seoul Regional Office, KOSHA, Seoul, Korea

(Received April 25, 2013; Revised June 13, 2013; Accepted June 26, 2013)

To investigate the effects of short-term exposure of beryllium on the human immune system, the proportion of T-lymphocytes such as CD3+, CD4+, CD8+, CD95, and NK cells, andthe proportion of B cells and TNFα level in peripheral blood and immunoglobulins in the serum of 43 exposed workers and 34 healthy control subjects were studied. External exposure to beryllium was measured by atomic absorption spectrometer as recommended by the NIOSH analytical method 7300. T lymphocyte subpopulation analysis was carried out with flow cytometer. The working duration of exposed workers was less than 3 months and the mean ambient beryllium level was 3.4 $\mu g/m^3$, 112.3 $\mu g/m^3$, and 2.3 $\mu g/m^3$ in molding (furnace), deforming (grinding), and sorting processes, respectively (cited from Kim *et al*., 2008). However, ambient beryllium level after process change was non-detectable (< 0.1 $\mu g/m^3$). The number of T lymphocytes and the amount of immunoglobulins in the beryllium-exposed workers and control subjects were not significantly different, except for the total number of lymphocytes and CD95 (APO1/FAS). The total number of lymphocytes was higher in the beryllium-exposed individuals than in the healthy control subjects. Multiple logistic regression analysis showed lymphocytes to be affected by beryllium exposure (odd ratio = 7.293; $p < 0.001$). These results show that short-term exposure to beryllium does not induce immune dysfunction but is probably associated with lymphocytes proliferation.

***Key words*:** Beryllium, Short-term exposure, T lymphocyte subpopulation, Immunoglobulins

INTRODUCTION

The lightweight metal element beryllium causes injury to lungs, skin, and other organs through direct chemical toxic effects and through its ability to induce beryllium antigen-specific sensitization and granulomatous lung disease (1). Nine cases of acute interstitial pneumonitis suspected to be acute beryllium disease were found at a factory making liquid metal alloy ingots consisting of Zirconium (Zr), Beryllium (Be), Nickel (Ni), Copper (Cu), Titanium (Ti) in Korea (2). We had conducted an epidemiological survey to inquire into the outbreak of acute interstitial pneumonitis. However, although this event was known to bea case of acute beryllium disease (3), no one knew the definite mechanism of the outbreak. Beryllium (CAS Registry No. 7440-41-7) is a very stable, extremely hard, and light metal element (atomic weight 9) in the earth and has several unique properties such as very high electronegativity and thermal conductivities. Therefore, beryllium are widely used in modern manufacturing of ceramics and plastics, electrical and electronic parts, telecommunication industries, nuclear products, X-ray machines and so on (4-6). Beryllium can enter a living body through inhalation, eating food or drinking water contaminated with beryllium and a very low amount can enter through skin contact with the metal. The respiratory tract in humans is a major target of beryllium inhalation exposure. Beryllium exposure poses considerable health risks for workers. Exposure to beryllium severely affects the lungs, liver, kidney, skeleton and lymph nodes (4,7) and causes a variety of diseases, including dermatitis, granulomatosis, pneumonia, chronic beryllium disease (CBD) and cancer (8). Workers who are occupationally short-term exposed to beryllium may show acute pneumonitis, but long-term exposure

Correspondence to: Ki-Woong Kim, Center for Occupational Health Research, Occupational Safety and Health Research Institute, KOSHA, #478 Munemi-ro, Bupyeong-gu, Incheon 403-711, Korea
E-mail: k0810@kosha.net

is more associated with berylliosis, in which granulomatous lesions develop in the lungs (9-12) and immunological diseases are also induced (8,13). However, research into immune dysfunction in short-term beryllium exposure is very rare and the mechanisms involved are not clearly understood. The 8-hours occupational exposure limit of 2 μg/m^3 for beryllium, recommended by the Korean Ministry of Employment and Labor in 2010, has been accepted because beryllium exposures below the threshold limit value (TLV) can also cause chronic beryllium disease in some people who have become sensitized to beryllium. Studies investigating the risks of beryllium sensitization and its related diseases have mostly considered long-term exposure to beryllium (14-16).

The objective of this study was therefore to investigate the relationship between short-term exposure to beryllium and its influence on human immune response.

MATERIALS AND METHODS

Subjects. Subjects examined comprised 43 workers who had been short-term exposed to beryllium at a manufacturingplant producing liquid metals containing beryllium, in Korea. The exposed workers included 36 men and 7 women aged between 22~54 years (30.9 ± 8.0) with the same frequency of both smokers and alcohol drinkers of 26/43 (60.5%). The subjects were exposed to beryllium during a 3 month period (36 days-88 days) and the exposure was terminated 1 month prior to the study commencing. The reason for taking a chance in terms of the incident exposure pause (1 month) of operation was only due to administrative order to stop the work process. The control subjects had never been occupationally exposed to hazardous including beryllium (Table 1). Thirty-four healthy controls were recruited from a university and their health status was assessed by interview. Those who had chronic illnesses including cancer were excluded from the study. This study was approved by the Institutional Review Board of Occupational Safety and Health Research Institute (COHR13-12). Written informed consent for the subjects in this study was obtained from all individuals.

Ambient monitoring. External exposure to beryllium was measured by atomic absorption spectrometer (Model AAnalyst 800, Perkin Elmer, NY, USA) as recommended by NIOSH analytical method 7300 (17), and the detection limit for beryllium was 0.08 μg/sample.

Table 1. Characteristics of the study subjects

	Exposed subjects (n = 43)	Controls (n = 34)
Age (years), mean ± SD	30.9 ± 8.0	30.6 ± 9.1
Gender, female (%)	7 (16.3%)	8 (23.5%)
Exposure duration (months)	< 3 months	non-exposure
No. of smokers (%)	26 (60.5%)	13 (38.2%)
No. of alcohol drinkers (%)*	26 (60.5%)	30 (88.2%)

*: $p < 0.05$.

Peripheral blood analysis. Hematological parameters including the number of white blood cells (WBC), red blood cells (RBC), lymphocytes, monocytes, neutrophils, eosinophils, basophils, hemoglobin and hematocrit were determined using Automated Hematology Analyzer (Sysmex SF 3000, TOA Medical Electronics Co, Kobe, Japan).

Immunological analyses. T lymphocyte subpopulation analysis in samples was carried out using flow cytometer, XL Coulter Epics (Coulter, Florida, USA) within 12 hrs of blood collection using the following sets of monoclonal antibodies: anti-T3 (CD3-FITC)/anti-B4 (CD19-PE), anti-T4 (CD4-FITC)/anti-3G8 (CD16+56)-PE, anti-T8 (CD8-FITC)/anti-S6F1 (CD11a-PE), CD95-FITC (APO1/FAS), TNFα-FITC and mouse IgG1-FITC/mouse IgG1-PE (negative control). One hundred μl of blood sample was incubated with each of the monoclonal antibodies for 15 min in the dark. All antibodies were used at optimal dilutions. After incubation, 2 ml of 1 × FACS lysing solution (Becton Dickinson, Cal, USA) diluted 10 times with distilled water, was added to each sample and the samples were mixed gently and incubated for 10 min in the dark to lyse the erythrocytes. Samples were centrifuged at 1,000 rpm for 5 min and the supernatant was aspirated. The pelleted cells were resuspended in 2 ml of phosphate buffered saline (PBS). After recentrifugation, the pelleted cells were resuspended in 0.5 ml PBS and analyzed for immunofluorescence on a flow cytometer. The number of the total lymphocytes of T (CD3+) cells, helper T (CD4+) cells, suppressor/cytotoxic T (CD8+) cells, APO1/FAS (CD95), NK cells and B (CD19+) cells were calculated by flow cytometer produced by XL Coulter Epics (Coulter, Fla, USA). Immunoglobulin (Ig) subfamilies such as IgA, IgE, IgG and IgM were analyzed by Behring Nephelometer Analyzer II (DADE Behring Co., IL, USA) using monoclonal antibodies such as rabbit anti-human IgG, IgA, IgEandIgM antibodies.

Statistical analysis. Statistical analysis was performed using SPSS V 12.0 software (SPSS Inc. USA).

Differences in the number of T lymphocyte subpopulations and the concentrations of serum immunoglobulin subfamilies between exposed workers and healthy controls were examined by Student's t-test. Differences were considered as significant when the p value was less than 0.05 ($p < 0.05$).

RESULTS

The demographics of the subjects are presented in Table 1. The working duration of exposed workers was shorter

Table 2. Ambient beryllium levels before and after the change of manufacture process

Working process	Mean ambient beryllium concentration ($\mu g/m^3$)	
	Before (range)[§]	After (range)
Molding (furnace)	3.4(0.4-4.8) (n = 3)	0.1(0.0-0.1) (n = 5)
Deforming (grinding)	112.3 (n = 1)	0.1(0.0-0.1) (n = 3)
Sorting	2.3(0.7-3.1) (n = 4)	0.1(0.0-0.1) (n = 5)

[§]: Cited from Kim *et al.* (2).

Table 3. Hematological parameters in study subjects

	Exposed subjects (n = 43) Mean ± SD	Controls (n = 34) Mean ± SD
WBC	7.45 ± 1.95	6.05 ± 1.40
RBC	4.91 ± 0.47	4.90 ± 0.44
Lymphocyte**	2.85 ± 0.79	1.95 ± 0.52
Neutrophil	3.58 ± 1.49	3.09 ± 1.11
Monocyte	0.80 ± 0.29	0.75 ± 0.22
Eosinophil	0.19 ± 0.15	0.21 ± 0.15
Basophil	0.04 ± 0.02	0.04 ± 0.02
Hemoglobin	15.35 ± 1.86	15.06 ± 1.32
Hematocrit	44.46 ± 4.64	44.54 ± 3.49

**$p < 0.01$, Units: WBC, Lymphocyte, Neutrophil, Monocyte, Eosinophil, Basophil; 10^3 cell/μl, RBC; 10^6 cell/μl, Hemoglobin; g/dl and Hematocrit; %.

than 3 months and the frequency of smokers in the exposed workers was higher than in the control, although this difference was not statistically significant. On the other hand, the frequency of alcohol consumption was significantly higher in control than in exposed workers ($p < 0.05$). The ambient beryllium level before and after manufacturing process change is shown in Table 2. In this study, we could not measure the beryllium levels before change to the working process because changed to working process by the workers' health problems. So, exposure levels before the change of the working process were cited from research report by Kim *et al*. (2). The beryllium level was 3.4 $\mu g/m^3$, 112.3 $\mu g/m^3$ and 2.3 $\mu g/m^3$ in the molding (furnace), deforming (grinding) and sorting processes, respectively (2). However, the ambient beryllium level after process change was non-detectable (less than 0.1 $\mu g/m^3$). The hematological parameter results are presented in Table 3. No statistically significant differences were observed for the hematological parameters between exposed subjects and controls. However, the number of lymphocytes was significantly higher in

Table 4. The number of T lymphocyte subpopulations and the level of serum immunoglobulin subfamilies in study subject

	Exposed subjects (n = 43) Mean ± SD	Controls (n = 34) Mean ± SD
T lymphocytes (per mm^3 whole blood)		
CD4+	1734.7 ± 433.9	1801.6 ± 190.9
CD8+	1232.1 ± 337.7	1188.4 ± 390.1
CD4+/CD8+ ratio	1.50 ± 0.51	1.94 ± 1.87
CD3+	3190.3 ± 583.9	3391.1 ± 398.1
CD95*	301.2 ± 128.3	393.2 ± 198.1
NK cell	947.6 ± 511.5	884.8 ± 387.7
B-cell	758.1 ± 302.2	704.4 ± 249.6
TNFα	77.8 ± 63.1	91.6 ± 95.6
Immunoglobulins (Ig) (mg/dl)		
IgA	245.4 ± 69.3	208.4 ± 70.4
IgE	178.7 ± 353.6	281.2 ± 530.7
IgG	1213.5 ± 222.2	1235.7 ± 214.5
IgM	143.4 ± 84.5	110.9 ± 47.6

*: $p < 0.05$.

Table 5. The number of T lymphocyte subpopulations and the level of serum immunoglobulin subfamilies according to working process

Variables	Working process	
	Molding (n = 3) Mean ± SD	Sorting (n = 4) Mean ± SD
Exposure levels		
Beryllium exposure, mean (range) ($\mu g/m^3$)	3.4 (0.4-4.8)	2.3 (0.7-3.1)
Cumulative exposure level, mean (range)	0.118 (0.126-1.44)	0.279 (0.147-0.939)
T lymphocytes (per mm^3 whole blood)		
CD4+	1737.9 ± 401.5	1724.6 ± 339.7
CD8+	1292.1 ± 321.2	1244.9 ± 341.7
CD3+	3237.2 ± 637.3	3069.5 ± 393.9
CD95*	300.4 ± 107.2	384.1 ± 137.3
NK cell	912.0 ± 599.2	1061.6 ± 283.2
Immunoglobulins (Ig) (mg/dl)		
IgA	251.6 ± 72.9	256.9 ± 59.3
IgE	190.9 ± 401.9	133.8 ± 181.1
IgG	1193.7 ± 227.7	1307.5 ± 198.4
IgM	147.4 ± 94.6	140.8 ± 59.4

Table 6. Interrelationship adjusted age, gender, smoking and drinking habit between beryllium exposure and lymphocyte subpopulations using multiple logistic regression analysis

Dependent variables	B	S.E	OR	95%CI	p value
Lymphocytes	1.987	0.601	7.293	2.245-23.695	0.001
CD3+	–0.821	0.545	0.440	0.151-1.279	0.132
CD4+	–1.161	0.584	0.313	0.100-0.983	0.047
CD8+	0.782	0.562	2.186	0.727-6.571	0.164
CD95+	–0.841	0.580	0.431	0.138-1.345	0.147
NK cell	0.466	0.577	1.594	0.514-4.944	0.419

OR, odds ratio; S.E, standard error; 95% CI, 95% confidence interval; NK cell, natural killer cell.

exposed subjects ($p < 0.01$) than in the controls. We determined the number of lymphocyte subpopulations such as CD4+, CD8+, CD3+, CD95 and NK cells as well as B-cells and tumor necrosis factor α (TNFα). No significant differences were also observed between exposed subjects and controls, except for the number of CD95 (APO1/FAS), which was decreased in exposed workers ($p < 0.05$) (Table 4). The number of T lymphocyte subpopulations and the level of serum immunoglobulin subfamilies according to cumulative exposure levelof working process were not also showed significant difference except grinding (only one sample) (Table 5). And multiple logistic regression analysis result shows CD95 (APO1/FASD) did not affected by beryllium exposure and only lymphocyte strongly affected by beryllium exposure (odd ratio = 7.293, $p < 0.001$) (Table 6).

DISCUSSION

Acute beryllium disease is considered to be an irritative chemical phenomenon and the disease has been associated with airborne beryllium concentrations > 100 μg/m^3(3). Beryllium particles created during metal machinery industry processes are mainly of submicron size and beryllium persists within the lungs of individuals many years after exposure has ceased, suggesting a failure to clear beryllium antigens from lungs (1). One of the major findings of this study is that short-term beryllium exposure does not immediately affect the immune system, but does have an effect on the early stages of immunosuppression and induces T cell-mediated inflammation (1). The number of T lymphocyte subpopulations, such as CD3+, CD4+, CD8+, NK along with IgA, IgG, IgE and IgM, were not significantly different between the exposed subjects and controls. Among the hematological parameters, the number of lymphocytes was significantly larger in exposed subjects than in controls ($p < 0.01$). On the other hand, a significant decrease in CD95 (APO1/FAS) was observed in exposed subjects. And our resultdid not show statistical differences of cellular and humoral immunity between molding process and sorting process according to cumulative exposure level. These results indicate that the cumulative effects are association with short-term exposure and by low body burden due to low level exposure (8).

Exposure to beryllium in workplaces can result in beryllium sensitization or granulomatous disorder (9). Beryllium acts as an antigen, which is presented by the antigen-presenting cells (APC) to a specific surface antigen receptor of the CD4+ T cells (18,19). As a result, beryllium accumulates the CD4+ T cells. Activation and accumulation of beryllium specific T cells triggers the production of cytokines such as interleukins (ILs), interferons (IFNs) and macrophage-activating factor (20). Also, CD4+ and CD8+ lymphocytes are proliferated upon exposure to beryllium (21).

However, in this study, these variables were not significantly different between the exposed subjects and the controls. The observations made in this research thus suggest that in short-term beryllium exposure there are no differences in terms of the abovementioned findings. Also, the reason for the workers' apparently unaffected immune systems is that beryllium is associated with delayed type hypersensitivity (DTH), for which it is difficult to eliminate the antigens effectively.

In the early stages before immune dysfunction is induced by antigens, ILs such as IL2, IL6 and IL10 are released (22,23) by the APC, and macrophages are activated by the released cytokines. The activated macrophages and released cytokines induce inflammatory disorder of the lungs during the immune reaction. Also, although the relationship between beryllium-induced apoptosis and inflammation in humans is not clear, beryllium could induce apoptosis in macrophages from chronic beryllium diseased bronchoalveolar cells. However, beryllium-stimulated adherent macrophage apoptosis was found to be independent of TNFα (24). In this study, we determined the number of CD95 (APO1/FAS) and TNFα. The number of CD95 (APO1/FASD) in exposed workers was smaller than that in non-exposed workers ($p < 0.01$) but multiple logistic regression analysis result shows CD95 (APO1/FASD) did not affected by beryllium exposure, and TNFα was not different between the two groups. In this study, the increased number of lymphocyte in exposed workers demonstrates possible alterations to immune system. Long-term exposure of beryllium up-regulates the expression of surface CD95 (APO1/FASD) (1) which is a cell-surface receptor belonging to the TNFα

receptor family of apoptosis-signaling molecules (25) on human lung macrophages. However, our research results performed with the short-term exposure showed different results with this. In exposed group, CD95 (APO1/FASD) showed a tendency to decrease and lymphocyte was increased. Therefore, it is impossible to check directly whetherthere is a possibility that short-term exposure can cause apoptosis. However, the increase of lymphocyte is associated with an increase of inflammatory response, and it may be explained as a result of the inflammatory response and the accompanying oxidative stress by exposure to beryllium (26). Eventually, inflammatory response associated with an increase in macrophage apoptosis, the probability is deemed sufficient. The various biological (i.e. age and gender) or environmental factors (i.e. medication, smoking, alcohol intake) have been taken into account as potential confounders, and were found to have no influence on the correlations found in this study.

Finally, we suggest that short-term exposure to beryllium does not induce immune dysfunction, but it is possible that beryllium-induced lymphocytes proliferation. The present study had some limitation. The sample size was relatively small, and not matched to age and gender between in exposed worker and controls. Also, exposure levels of beryllium were not wide enough to find significant correlations between exposure levels of beryllium and immune system. To clarify the effects of beryllium on immune parameters, further studies including high and low beryllium concentrations and exposure durations are needed.

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