A Study on the Beryllium Lymphocyte Transformation Test and the Beryllium Levels in Working Environment

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Abstract: The relationship between airborne concentration of beryllium in the working environment and workers' beryllium lymphocyte transformation test (Be-LTT) values was examined based on data obtained from a four-year survey (1992-1995) conducted at beryllium-copper alloy manufacturing factories. This study showed that the T cells of workers continuously exposed to beryllium of more than 0.01 μg/m³ could be activated and that the cell-mediated immune response of workers could be promoted. On the other hand, the Be-LTT of workers exposed to beryllium levels of less than 0.01 μg/m³ was shown to be unaffected by beryllium. These findings suggest that beryllium sensitization is not manifested when level of beryllium in working environment are less than 0.01 μg/m³. Therefore, in such cases workers do not develop Chronic beryllium disease (CBD). We concluded that the Be-LTT can be applied as a medical indicator to detect the development of CBD.

Key words: Beryllium, Chronic beryllium disease, Lymphocyte transformation test, Beryllium-copper, Beryllium-copper alloy, T cell

Introduction

The development of beryllium diseases, especially chronic beryllium disease (CBD), may be closely linked with immunologic allergic response of workers exposed to beryllium¹-³. To prevent CBD, it is necessary to clarify how CBD develops and to take the necessary preventive measures. To answer the question of how CBD develops, many experimental studies have been conducted⁴-⁶. As a result, the mechanism of development of CBD is almost understood. A few points remain to be clarified. In particular, no clear explanation has yet been given of how beryllium sensitization is triggered by exposure to beryllium (Be), and how this leads to the development of CBD.

Measures to prevent the development of CBD at the workplace have been remarkably improved in the last decade. Thus, the improvement of the working environment and the management of workers' health care have remarkably decreased the incidence of CBD. However, a few cases of CBD development have been reported in some countries where working environment has been carefully managed and workers' health care have been strictly monitored⁷-⁹. On the other hand, there are a few reports on the relationship between airborne concentrations of beryllium in the working environment and the actual conditions of the immune system of workers exposed to beryllium. To examine the immune reaction to beryllium, many methods such as the beryllium skin test, the
macrophage migration inhibition test, the lymphocyte ratio in the blood, immune-globulin levels in the serum, and the lymphocyte transformation test have been applied. Of these the beryllium lymphocyte transformation test (Be-LTT) may be the easiest to use to evaluate hypersensitivity of individuals exposed to beryllium\(^6\). The lymphocyte transformation test is one of the tests conducted \textit{in vitro} for T-lymphocyte reactivity to antigens. When sensitized lymphocytes are cultured in the presence of the appropriate antigen, they respond by being transformed into dividing blast cells. This is measured by assaying the uptake of tritium thymidine into the cells. It is known as the Be-LTT when beryllium is used \textit{in vitro} as an antigen\(^{10,11}\).

In this paper, we report on a four-year survey (1992–1995) conducted on airborne concentrations of beryllium in the work environment and a beryllium lymphocyte transformation test in two beryllium-copper alloy manufacturing factories.

**Materials and Methods**

**Worksites and workers**

Two beryllium-copper (Be-Cu) alloy manufacturing factories designated as A and B were examined. Work environment of the A factory was subdivided into a Be-Cu alloy process (A-1), a Be-Cu metal mold manufacturing process (A-2) and the processes without exposure to Be, while that of the B factory was divided into a Be-Cu cold rolling, drawing and heat-treatment process (B-1), a Be-Cu slitting treatment process (B-2) and the other processes without Be exposure.

The number of workers who engaged in the A-1, A-2, B-1 and B-2 process during the 4 years was 24, 25, 6 and 28, respectively, and their corresponding mean age was 47 (range 19–58), 50 (range 32–59), 43 (range 39–48) and 46 (range 23–59) in the year 1995. Mean length of exposure to Be was 10.0 years (range 1.0–25.1), 16.0 (range 3.0–30.0), 16.0 (range 6.0–28.1) and 11.0 (range 2.0–31.1), respectively. All the workers were males.

During the surveying period, 159 workers with age of 21 ± 3 years (M ± SD) were newly recruited in the worksites without exposure to Be in the A and B factories. They did not have prior exposure to Be or any evidence of beryllium diseases. Be-LTT measurements were conducted at the time of new employment.

**Measurement of beryllium levels in working environments**

The airborne Be concentrations in each process were determined twice a year by the area sampling method\(^{12}\). The number of the sampling spots at A-1, A-2, B-1 and B-2 process during the four-year surveying period was 56, 41, 16 and 8 points, respectively. Airborne dust containing Be in the working environment was collected on a filter paper of a high volume air sampler (Open Face Model HVC-500, Sibata, Japan) and then digested with nitric acid and hydrogen peroxide. The digested solution was concentrated by heat and then diluted with ion-exchange water to a fixed volume. The Be concentrations were measured with an atomic absorption spectrophotometer equipped with a graphite furnace (Model Z-8000, Hitachi, Japan).

**Beryllium lymphocyte transformation test**

Lymphocytes were separated from the peripheral blood of workers by the Ficol-Conray 400 method\(^{13}\). After washing with phosphate-buffer saline (PBS, pH 7.4: Gibco Lab., USA), lymphocytes were suspended in an RPMI 1640 cell-culture medium (Gibco, Lab., USA). Then the autoplasma was mixed at a ratio of 1:4 to prepare a lymphocyte suspension of 10\(^6\) cells/ml. To a microtiter plate, 200 µl of the lymphocyte suspension and 10 µl of a BeSO\(_4\) solution (adjusted to concentrations of 1/100, 1/500, and 1/1000 from 2% BeSO\(_4\)) as the antigen were added. After incubating for 72 hr in a CO\(_2\) incubator at 37°C, 9.25 kBq/well of \(3H\)-thymidine \((3H-TdR)\) was added and the mixture was incubated for 16 hr under the same conditions.

Using an automatic cell harvester (LM101, Laboscience, Japan), lymphocytes were gathered on a glass fiber filter (GF/C, Whatman, UK). After the filter was dried, the amount of \(3H\)-TdR taken up into the lymphocytes was measured in counts per min (cpm) using a scintillation counter (LSC-653, LKB Co, Finland).

Tests were conducted on triplicate and the results were expressed as a stimulation index (SI\%) calculated by using following formula:

\[
SI\% = \frac{\text{mean cpm of cultures with beryllium}}{\text{mean cpm of cultures without beryllium}} \times 100
\]

The mean cpm was derived from a series of triplicates. As for the concentration of BeSO\(_4\), which was used as the antigen, the final concentration was adjusted to 1.72 × 10\(^{-6}\), 3.44 × 10\(^{-7}\), 1.72 × 10\(^{-7}\) M showed the strongest blast transformation.

All data were compared using the Student’s t test.
Results

Beryllium levels in working environments

Table 1 shows the geometric mean value and the range of airborne Be concentration at each of the four manufacturing processes. The Be level was the highest at the A-1 process (0.16–0.26 µg/m³) than at any other processes. It was, however, worthy to note that there were much differences in the Be level among the sampling spots within the A-1 process. Thus, a very high Be level of 1.85 µg/m³ was tentatively noted. The Be levels in the A-2 process ranged from 0.01 to 0.02 µg/m³. In the B-1 process, the Be levels in 1993 were approximately as high as those in the A-1 process, but the levels decreased year after year, indicating 0.03 µg/m³ in the 1994 and 1995 surveys. The reason for this was that the improvement of local exhaust systems and cleaning of deposited particulate materials were carried out in the B-1 process in 1994. The Be levels in the B-2 process were found to be less than 0.01 µg/m³.

All the airborne Be concentrations in the A and B factories in the four-year survey were found to be less than 2.0 µg/m³, the administrative level for beryllium set by Ministry of Labour, Japan.

Beryllium lymphocyte transformation test

1) Be-LTT of workers exposed to beryllium

Table 2 shows the Be-LTT results with mean ± SD and range of SI% and number of workers giving an SI value of more than 200%. Arranging each manufacturing process by starting with the highest SI%, the A-1 process was the first (133 ± 61%), followed by the B-1 process (133 ± 51%), A-2 process (120 ± 57%) and B-2 process (100 ± 21%). The prevalence of an SI value of more than 200% was 11% (8 in 73 determinations for 24 workers) at the A-1 process, 8% (7 in 93 determinations for 25 workers) at the A-2 process, 6% (1 in 18 determinations for 6 workers) at the B-1 process. All of 56 determinations for 28 workers at the B-2 process were found to be less than 200% SI.

Chronological observations on changes in the Be-LTT of the same workers revealed that all of the cases giving an SI value of more than 200% in A-1 (8 determinations) and B-1 process (one determination) were not the results by the same workers. For example, one case in the A-1 process gave an SI value of 262 in 1992, 80% in 1993, 140% in 1994 and 187% in 1995. On the other hand, three workers in the A-2 process gave an SI value of more than 200% in two or three times during the four years: the first worker giving 249% in 1992 showed 287% in 1993, 125% in 1994 and 206% in 1995, respectively. The second worker giving 339% in 1992 showed a decrease in 1993 and 1994, 193% and 70%, respectively but showed an increase to showing 400% in 1995. The third worker giving an SI value of 107% in 1992 and 121% in 1993 showed 206% in 1994 and 269% in 1995. Thus the actual number of workers giving an SI value of more than 200% was 12 out of 83 workers.

Comparing the SI% of the Be-LTT between processes, a statistical significance was revealed between that of A-1 and B-2 process, and A-2 and B-2 process, respectively (P<0.05) in 1993, and also the A-1 and B-2 process (P<0.001), A-2 and B-2 process (P<0.01), and the B-1 and B-2 process (P<0.05) in the total number of workers surveyed from 1992 to 1995.

2) The Be-LTT of new workers

The individuals were tested for the Be-LTT immediately after being employed at the A and B factories. Mean ± SD of SI % was 105 ± 26% for 137 workers with a mean age of 22 in 1992, 95 ± 17% for 9 workers with mean age of 18 in 1993, 121 ± 27% for 7 workers with mean age of 19, and 137 ± 23% for 6 workers with mean age of 19. The averaged SI value was 112 ± 23% for a total of 159 workers at the time of employment. This value was found to be significantly higher than that in the B-2 process (P<0.05). Two workers out of the total number of 159 exhibited an SI value of more than 200%.

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Discussion

Hanifin et al. and Hartog et al. reported that the Be-LTT was useful for the diagnosis of CBD. It has been recognized that the positive reaction of the Be-LTT in CBD cases was higher than that in healthy workers exposed to beryllium, workers not exposed to beryllium, and other individuals with pulmonary diseases. Williams and Williams made the Be-LTT method more reliable by examining the concentration of adding autoplasm or beryllium in the culture system in vitro. As a result, this method is currently being used to diagnose CBD cases.

We conducted the four-year survey to clarify the relationship between beryllium levels in working environment and the Be-LTT value of workers in the work environment of Be-Cu alloy manufacturing processes. It was further examined whether the Be-LTT value of workers exposed to beryllium could be used to check the immune system of workers, and could be applied as a medical index to detect the development of CBD.

The present survey showed that airborne concentrations of beryllium in working environment were positively related to changes in the Be-LTT value of workers. More specifically, the Be-LTT values of workers were found to increase, as the beryllium levels in the work environment increased. This relationship was especially obvious in the working environments where beryllium levels were higher than 0.01 µg/m³. The present results suggest that T cells of workers continuously exposed to beryllium of more than 0.01 µg/m³ may be activated, and the cell-mediated immune response of workers may be promoted.

On the other hand, the Be-LTT value of workers (B-2 workers) exposed to beryllium of less than 0.01 µg/m³ were found to be unaffected. This suggests that beryllium sensitization is not manifested when airborne concentration of beryllium were less than 0.01 µg/m³ in working environment. Therefore, such beryllium

Table 2. Stimulation index (SI%) of a beryllium lymphocyte transformation test for workers in the beryllium-copper alloy manufacturing processes in the years from 1992 to 1995

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<tbody>
<tr>
<td>A-1 process</td>
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<tr>
<td>No. of workers examined</td>
<td>15</td>
<td>16</td>
<td>18</td>
<td>24</td>
<td>73</td>
</tr>
<tr>
<td>SI% M ± SD</td>
<td>117 ± 52</td>
<td>133 ± 58*</td>
<td>116 ± 37</td>
<td>159 ± 75</td>
<td>133 ± 61***</td>
</tr>
<tr>
<td>Range</td>
<td>72–262</td>
<td>80–243</td>
<td>63–211</td>
<td>73–388</td>
<td>63–388</td>
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<tr>
<td>SI &gt;200%a</td>
<td>1 (7%)</td>
<td>2 (15%)</td>
<td>1 (6%)</td>
<td>4 (17%)</td>
<td>8 (11%)</td>
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<tr>
<td>A-2 process</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>No. of workers examined</td>
<td>19</td>
<td>24</td>
<td>25</td>
<td>25</td>
<td>93</td>
</tr>
<tr>
<td>SI% M ± SD</td>
<td>132 ± 66</td>
<td>117 ± 48*</td>
<td>98 ± 33</td>
<td>135 ± 71</td>
<td>120 ± 57**</td>
</tr>
<tr>
<td>SI &gt;200%a</td>
<td>2 (11%)</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
<td>3 (12%)</td>
<td>7 (8%)</td>
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<tr>
<td>B-1 process</td>
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<td>No. of workers examined</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>SI% M ± SD</td>
<td>135 ± 81</td>
<td>120 ± 32</td>
<td>143 ± 28</td>
<td>133 ± 51*</td>
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<tr>
<td>SI &gt;200%a</td>
<td>1 (17%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (6%)</td>
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<td>B-2 process</td>
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<tr>
<td>No. of workers examined</td>
<td>28</td>
<td>28</td>
<td>28</td>
<td>56</td>
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<tr>
<td>SI% M ± SD</td>
<td>95 ± 24</td>
<td>106 ± 15</td>
<td>100 ± 21</td>
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</tr>
<tr>
<td>Range</td>
<td>59–184</td>
<td>74–145</td>
<td>59–184</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SI &gt;200%a</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
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a) Number of workers and its percent giving an SI value of more than 200%. Significant difference comparing with B-2; ***p<0.001, **p<0.01, *p<0.05. Range (minimum–maximum).
levels are not considered to cause CBD. In other words, the cell-mediated immune response of workers exposed to beryllium seems to be causally related to the beryllium levels in the working environment.

Next, we examined the beryllium sensitization on the basis of our four-year survey. Williams et al. reported that the beryllium sensitization appeared at a Be-LTT value of more than 200%, while normal people and other patients without CBD gave a negative Be-LTT response. In our survey, 12 out of 83 workers exhibited a Be-LTT value of more than 200%. However, it is not clear whether the case of all these workers resulted from the beryllium sensitization. Eight workers among the workers in the A-1 process exhibited a positive response only once in the four years survey. One worker among the workers in the B-1 process showed much the same phenomenon as the eight cases in the A-1 process. On the other hand, three workers among the workers in the A-2 process showed positive responses two or three times during the four-year surveying period, making these workers different from the cases in the A-1 and B-1 processes. It is likely that the three workers in the A-2 process are sensitized to beryllium. However, we cannot definitely conclude that they have an increased risk of developing CBD. This investigation demonstrates that the beryllium sensitization may occur under occupational conditions where beryllium levels are less than 2.0 µg/m³, the administrative level for beryllium set by Ministry of Labour, Japan.

Next, a comparison of the Be-LTT values of workers with potential exposure to less than 0.01 µg/m³ beryllium and those of workers without beryllium exposure showed that the former were lower than the latter. Naturally, the latter showed the values of more than 100%, since beryllium enhances DNA synthesis in lymphocytes treated with beryllium in vitro. However, we cannot present a satisfactory explanation for why the lymphocyte activity of workers continuously exposed to a very small amount of beryllium was decreased compared to that of workers not exposed to beryllium. We think that this phenomenon may be explained by exposure of the workers' immune system to a very small amount of beryllium. Moreover, two cases of the latter group (159 persons) gave an SI value of more than 200%; however, this may be related to some hereditary factor and the sensitivity of particular individuals to beryllium, as it is unlikely that they would have been exposed to beryllium. Thus, it is believed that some individuals are particularly sensitive to beryllium.

In conclusion, the present survey demonstrated that the Be-LTT values of workers exposed to beryllium levels of more than 0.01 µg/m³ were significantly higher than those of workers exposed to less than 0.01 µg/m³. Furthermore, some workers in the A factory may have exhibited the beryllium sensitization. It should also be noted that although beryllium sensitization is an important indicator of CBD, the sensitization does not always develop into a beryllium disease. It can be emphasized on the basis of the present survey that it is very important to regularly check airborne concentrations of beryllium in working environment, as well as the Be-LTT value of workers, at least once a year in order to prevent the development of CBD.

References

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