Immunological effects of occupational exposure to metallic mercury in the population of T-cells and NK-cells†

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This paper presents a study of the counts of lymphocytes, (CD3+)T-cells, (CD4+)T-helper and (CD8+)T-suppressor and (CD16+)NK-cells in the peripheral blood of 101 males with a history of occupational exposure to metallic mercury vapo(u)rs (Hg⁰) and in 36 males without such exposure. These workers were divided depending on the duration of exposure: 37 males with a short-term history of exposure to Hg⁰ (up to 10 years) and 64 males with a history of long-term exposure (10 to 37 years). For the determination of T-cell populations monoclonal antibodies were used in indirect immunofluorescence tests. The time weighted average of mercury concentrations in air was 0.028 mg m⁻³. Mercury concentration in the urine of the exposed subjects ranged from 20–260 μg dm⁻³, and in blood it was from 4 to 72 μg dm⁻³. Stimulation of the T-cell line was noted as evidenced by increased numbers of (CD3+)T-cells, (CD4+)T-helper and (CD8+)T-suppressor cells in the workers with < 10 or > 10 years’ exposure to Hg⁰. Lower increase count of (CD3+)T-cells and (CD4+)T-helper cells than (CD8+)T-suppressor cells was the cause of decreased values in the (CD3+)T/(CD8+)T-suppressor ratio and (CD4+)T-helper/(CD8+)T-suppressor ratio in the workers with < 10 or > 10 years’ exposure. Moreover, no changes were observed in the T-cell populations between workers with < 10 and those with > 10 years’ exposure. In addition, statistical analysis of the effects of age and duration of exposure to Hg⁰ on the studied immunological parameters indicates that exposure duration may affect some of the values. These quantitative changes of T-cell population as well as changes of the (CD3+)T/(CD8+)T-suppressor and (CD4+)T-helper/(CD8+)T-suppressor ratio have been proposed as immunological indicators of exposure to Hg⁰, which can be used for monitoring and to explain the origin of autoimmunity disorders induced by metallic mercury.

Keywords: Metallic mercury vapo(u)rs; occupational exposure; immunity; lymphocytes; (CD3+)T-cells; (CD4+)T-helper; (CD8+)T-suppressor; (CD16+)NK-cells; monoclonal antibodies

The immunotoxicology of mercury compounds, especially metallic mercury, is important clinically. Some heavy metals such as mercury and lead can enhance humoral immunity, mainly antibody production with autoimmunity responses, suppress cell-mediated immunity and interfere with host defence against pathogens by direct action on cells of the immune system. The two major cell types responsible for the regulation of cell-mediated immunity are macrophages and (CD4+)T-helper (Th) cells of the Th1 subset. The Th1 subset produces IL-2 and IFN-γ upon activation; whereas, the Th2 subset produces IL-4, IL-5, IL-6 and IL-10 which preferentially enhance humoral immunity (Lawrence,1 Zelikoff et al.). Immunoregulatory networks are also based on a delicate balance between T-helper and T-suppressor cells.

The effect of mercury on the immune system has been studied mainly in laboratory animals, and it has been found that methylmercury inhibits both humoral and cell-mediated immune reactions. After administration to mice, mercury causes a transient atrophy of thymus cortex and lymph follicles (Hirokawa and Hayashi²), and reduces the number of NK-cells (Ilback³ and DNA synthesis of mitogen-stimulated lymphocyte cultures (Nakatsuru et al.). HgCl₂ stimulates DNA synthesis in lymphocytes and thymocytes (Nordlind⁶) and acts as a T-cell dependent polyclonal activator of B-cells (Ensault et al., Hultman and Enestrom,³³ Pusey et al.). Mercury can primarily activate murine T lymphocytes to transformation and proliferation in vitro. In vivo administration of HgCl₂ causes autoimmune manifestations in rats and mice (Jiang and Moller.¹⁰) Susceptible Brown–Norway (BN) rats exhibit a (CD4+)T-cell dependent polyclonal activation of B cells, the total number of T-cells increasing very rapidly but in contrast, Lewis (LEW) rats are resistant and develop an immunosuppression mediated by (CD8+)T-cells recruited by (CD4+)T-cells (Fillion et al.) HgCl₂ in certain concentrations also acts as a stimulator of human lymphocytes in cultures, increasing the production of lymphokines and DNA synthesis (Nordlind, Holst and Nordlind, Perlingeiro and Queiroz, HgCl₂ (up to 1000 ng) and methylmercury (up to 100 ng) impairs autoimmunity (Shenker et al.).³³) Little data is available on the effects of mercury vapo(u)rs on the human immune system (Izdebska-Szymona and Kopćel-Szlezak,¹⁵ Lawrence,¹ Moszczyński et al.,¹⁶ Zelikoff et al.), especially during long occupational exposure. Mercury has been shown to affect lymphocyte function and to modulate immune reactivity (Bigazzi, Michaelson et al.)⁹) The effects of exposure to mercury have been linked to autoimmune disorders, mainly types II and III allergic reactions, either by acting as a hapten or altering the antigenicity of cellular proteins (Perlingeiro and Queiroz, Zelikoff et al., Queiroz et al.)³³) Low mercury vapour concentrations during occupational exposure can stimulate immunity. In 41 men exposed to Hg⁰ vapo(u)rs elevated levels of serum immunoglobulins IgA, IgM and serum acute phase proteins were observed (Bencze et al.).²²) The influence of low exposure to inorganic mercury on the T-cell population was examined only in 36 workers occupationally exposed to mercury vapo(u)rs (Langworth et al.)²²) The white blood cell differential counts, serum autoantibodies and in vitro production of the cytokines [IL-1, IL-6 and tumor necrosis factor alpha (TNFα)] as well as CD3, CD4, CD8, CD14 and CD25 lymphocyte surface markers were determined. All of the immunologic parameters were within normal ranges. However, in the group sensitized to mercury, there was a reduction of the

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in vitro production of both TNFα and IL-1 compared with the reference values. The relatively small size of the study group and low-level exposure to mercury vapours limited the general significance of these results (Langworth et al.22).

The purpose of the present study was to find out whether or not the biological effects of higher occupational exposure to metallic mercury are reflected in the quantitative changes of the populations of T-cells and NK-cells.

Experimental

The study was carried out in 37 males, aged 21 to 54 years old ($X = 30.9 \pm 8.0$) with occupational exposure to mercury vapours ($Hg_0$) for up to 10 years (7 months to 9 years) ($X = 4.2 \pm 2.8$) and 64 males aged 28 to 60 years ($X = 47.0 \pm 7.0$) with a history of exposure from 10 to 37 years ($X = 22.6 \pm 7.3$). The subjects worked in chloride production by the electrolytic method in three 8 h shifts. Of these, 25 (68%) and 39 (61%), respectively, were smokers.

The control group comprised 36 males aged 28–55 years ($X = 46.0 \pm 8.7$) not exposed to any chemical compounds or harmful physical influences; 29 (80.6%) were smokers. They worked in a three-shift system in the same plant. All workers were subjected to medical examination for ruling out those with diseases which might have affected their immune status. Alcoholics, and those regularly taking drugs and convalecents after infectious diseases were disqualified.

The air for determination of metallic mercury concentrations was taken with fixed samplers from area samples (Principles of Air Sampling in Work Environment and Interpretation of Results, Polish Committee of Norms, PN 89 Z 04008/07). The air sampling was done at two different points in this work place, according to the principles of stationary measurements (Gromiec and Rogaczewska.23) The air was sampled for 20 min using a pump (the speed of sampling was 0.18 $m^3 h^{-1}$), according to the Polish Committee of Norms, PN 69/C 13048. Air samples were taken in two different work places 6 times on morning work shifts, during the work time of subjects exposed to mercury vapour. Aspirated air samples were flown to the reaction vessel, which contained 50 cm$^3$ of 1% KMnO$_4$ solution in 10% H$_2$SO$_4$. The aspirated metallic mercury was oxidized to Hg$_2^+$. After 20 min of air sampling, the excess of KMnO$_4$ was reduced using hydroxylamine hydrochloride. Thereafter, Hg$_2^+$ ions were reduced to metallic Hg$_0$, by using tin(II) chloride (SnCl$_2$) and then Hg$_0$ was removed by aeration and determined using an atomic absorption spectrometer (Coleman Mercury Analyzer Mas-50, Perkin-Elmer, Norwalk, CT, USA, $\lambda = 253.7$ nm). The detection limit of the analytical procedure for determination of mercury in urine was 10 $\mu g$ dm$^{-3}$ and in blood was 4 $\mu g$ dm$^{-3}$. The ‘maximum permissible concentration’ (MPC) of metallic mercury is 0.05 $mg dm^{-3}$. The mercury content in urine and blood was determined by a method similar to that for Hg determination in air samples (AAAS). The urine and blood samples were mineralized with KMnO$_4$ in acidic medium and the excess of the oxidizing agent was reduced with hydroxylamine hydrochloride. Thereafter the samples were reduced with SnCl$_2$ in the aeration vessel and the liberated mercury was removed by aeration. Urine was obtained between 8:00 and 11:00 am for the determination of total mercury. The mercury concentration was expressed in $\mu g$ dm$^{-3}$ of urine of specific mass 1.024 g. Collection of urine samples was made carefully to avoid external contamination. The mercury concentration in urine has maximum values during evening and morning hours and has minimum values in the afternoon (Piotrowski et al.24 Wallis and Barber25) and therefore sampling time was during the morning hours. Blood samples for mercury determination were obtained from the veins of fasting subjects during the morning hours to avoid external contamina-

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Leucocytes/10$^9$dm$^{-3}$</th>
<th>Total (CD3+)T-cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>an$^*$</td>
<td>%</td>
</tr>
<tr>
<td><strong>Control (C), n$^*$ = 36</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$X = 6.47$</td>
<td>2.05</td>
<td>32.8</td>
</tr>
<tr>
<td>$\pm s = 1.59$</td>
<td>0.56</td>
<td>7.0</td>
</tr>
<tr>
<td><strong>Exposed to Hg$_0$ for less than 10 years, n$^*$ = 37</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A$^a$</td>
<td>AAA$^{a,b}$</td>
<td>AAA$^a$</td>
</tr>
<tr>
<td>$X = 7.12$</td>
<td>3.00</td>
<td>43.1</td>
</tr>
<tr>
<td>$\pm s = 1.63$</td>
<td>0.75</td>
<td>10.1</td>
</tr>
<tr>
<td><strong>Exposed to Hg$_0$ for 10 to 37 years, n$^*$ = 64</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A$^a$</td>
<td>AA$^a$</td>
<td>AA$^b$</td>
</tr>
<tr>
<td>$X = 7.27$</td>
<td>2.86</td>
<td>40.0</td>
</tr>
<tr>
<td>$\pm s = 1.92$</td>
<td>0.78</td>
<td>7.7</td>
</tr>
<tr>
<td><strong>All exposed to Hg$_0$, n$^*$ = 101</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A$^a$</td>
<td>AAA$^{a,b}$</td>
<td>AA$^a$</td>
</tr>
<tr>
<td>$X = 7.22$</td>
<td>2.91</td>
<td>41.1</td>
</tr>
<tr>
<td>$\pm s = 1.81$</td>
<td>0.77</td>
<td>8.8</td>
</tr>
</tbody>
</table>

In all cases, the absolute number ($\times 10^9$ cells per dm$^3$): $n$, Number of workers. * After logarithmic conversion. Statistical significance (C: I or C: II or C: III). + Analysis of variance (ANOVA): AAA = $p < 0.001$; AA = $p < 0.01$; A = $p < 0.05$. ¶ (Cochran-Cox test or Student’s t test): XXX = $p < 0.001$; XX = $p < 0.01$; X = $p < 0.05$.

[No statistical significances were observed in the leucocytes and T-cell populations between workers (I) with <10 and (II) those with >10 years exposure to metallic mercury.]
were subjected to normal distribution analysis by the Shapiro–Wilk Gaussian decomposition test. Thereafter, the results indicating normal distribution, without or after logarithmic conversion, were subjected to statistical analysis by a Cochrane–Cox $t$ test or Student’s $t$ test. Analysis of variance (ANOVA) amongst all groups and comparison between the exposed groups were also calculated.

In addition, the correlation coefficient was calculated for the analysed immunological parameters and the influence of the age of workers and the duration of exposure to metallic mercury on these parameters was assessed.

Results and discussion

Air analyses conducted regularly since 1983 have demonstrated that the maximum permissible concentration was exceeded by the mean Hg$^0$ values particularly in 1983 (1.8 times), in 1984 (1.5 times), and in 1965, 1978 and 1980 (1.1 times). In relation, however, to the maximum Hg$^0$ concentrations in these years the MPC was exceeded 6.5; 6.0; 3.8; 4.2; 2.8 times, respectively. The shift time weighted average (TWA) of mercury determined was 0.028 mg m$^{-3}$.

All mercury exposed and non-exposed workers of the control group were regarded as healthy. The socioeconomic status of all workers in this study was similar. The urine mercury level of the exposed workers ranged from 20 to 240 mg dm$^{-3}$ ($\bar{X} = 81.4 \pm 60.9$) and in the blood from 4 to 72 mg dm$^{-3}$ ($\bar{X} = 18.3 \pm 15.0$). The levels of mercury in urine and in blood in the control group were regarded as healthy. The socioeconomic status of all workers exposed for < 10 years or by 16% ($p < 0.001$) in the workers exposed for > 10 years. The lower increase in count of (CD4+)T-cells in the workers exposed for < 10 years or by 25% ($p < 0.01$) in the workers exposed for > 10 years. The lower increase in count of (CD4+)T-helper cell than (CD8+)T-suppressor cell population caused the decreased value of the (CD3+)T/(CD8+)T-suppressor ratio by about 18% ($p < 0.05$) in the workers exposed for < 10 years or by 25% ($p < 0.01$) in the workers exposed for > 10 years. The lower increase in count of (CD4+)T-helper cell than (CD8+)T-suppressor cell population was the cause of the decreased value in the (CD4+)T/ (CD8+)T-suppressor ratio by about 18% ($p < 0.05$) in the workers exposed for < 10 years or by 16% ($p < 0.05$) in the workers exposed for > 10 years. No changes were observed in the T-cell populations between workers exposed for < 10 years and those exposed > 10 years (Tables 1 and 2). No statistically significant changes were observed in the T-cell populations between workers with up to 10 years exposure and those with > 10 years exposure. Occupational exposure to mercury caused a fall in percentage (CD16+)NK-cells but the absolute count of these cells in the exposed subjects was not different from that in non-exposed subjects.

No statistically significant correlation was observed in a group of all workers exposed to Hg$^0$ between their age and any other analysed parameters (correlation coefficient, $r$, was between $-0.11$ and 0.10). In addition, statistical analysis of the effects of age and duration of exposure to metallic mercury on

### Table 2 Effects of occupational exposure to metallic mercury on the population of T-cells, NK-cells, (CD3+)T/(CD8+)T-suppressor and (CD4+)T-helper/(CD8+)T-suppressor ratio

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>(CD4+)T-help</th>
<th>(CD8+)T-suppressor</th>
<th>(CD16+)NK</th>
<th>(CD3+)/CD8+</th>
<th>(CD4+)/CD8+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>an*</td>
<td>%</td>
<td>an*</td>
<td>%</td>
<td>an*</td>
</tr>
<tr>
<td>Control (C), n$^*$ = 36—</td>
<td>X</td>
<td>8.4</td>
<td>41.30</td>
<td>0.47</td>
<td>46.0</td>
</tr>
<tr>
<td>Exposed to Hg$^0$ for less than 10 years, n$^*$ = 37—</td>
<td>AAA$^{19}$</td>
<td>AAA$^{19}$</td>
<td>AAA$^{3}$</td>
<td>AAA$^{3}$</td>
<td>A$^{19}$</td>
</tr>
<tr>
<td>I</td>
<td>X</td>
<td>1.34</td>
<td>42.90</td>
<td>0.02</td>
<td>30.82</td>
</tr>
<tr>
<td>II</td>
<td>X</td>
<td>1.42</td>
<td>43.52</td>
<td>0.03</td>
<td>29.56</td>
</tr>
<tr>
<td>Exposed to Hg$^0$ for 10 to 37 years, n$^*$ = 64—</td>
<td>AAA$^{19}$</td>
<td>AAA$^{19}$</td>
<td>AAA$^{3}$</td>
<td>AAA$^{3}$</td>
<td>AA$^{19}$</td>
</tr>
<tr>
<td>I</td>
<td>X</td>
<td>1.24</td>
<td>9.58</td>
<td>0.33</td>
<td>7.87</td>
</tr>
<tr>
<td>II</td>
<td>X</td>
<td>1.27</td>
<td>43.31</td>
<td>0.02</td>
<td>29.27</td>
</tr>
</tbody>
</table>

* an, Absolute number ($\times 10^{9}$ cells per dm$^{-3}$). $^*$ n, Number of workers. $^+$ After logarithmic conversion. Statistical significance (C : I or C : II or C : III). $^\dagger$ Analysis of variance (ANOVA): AAA = $p < 0.001$; AA = $p < 0.01$; A = $p < 0.05$. $^\ddagger$ Cochrane–Cox $t$ test or Student’s $t$ test: XXX = $p < 0.001$; XX = $p < 0.01$; X = $p < 0.05$. [NB No statistical significances were observed in the leucocytes and T-cell populations between workers (I) with < 10 and (II) those with > 10 years exposure to metallic mercury.]
The studied immunological parameters indicate that exposure duration may affect some of the values (Figs. 1–6). The response surfaces show that, in particular, the percentage of the total lymphocytes (Fig. 1) and the (CD4+)T-helper cells (Fig. 3) was greatly affected by exposure to metallic mercury. The response surfaces in Figs. 5 and 6 show duration of exposure dependent decreases in (CD3+)/T/(CD8+)/T-suppressor ratio and (CD4+)/T-helper/(CD8+)/T-suppressor ratio.

The mechanism of mercury action on the human lymphocytes has only been studied a few times previously. It has not been established whether or not during occupational exposure mercury accumulated in lymphocytes. X-ray analysis by energy dispersion and wave dispersion spectrometers demonstrated that in certain diseases, e.g., laryngeal carcinoma, the amount of Hg and also Pb, Cl, Bi, Mo, Cd, Ru, Te, Sr, W and Re increases in circulating lymphocytes (Pilch et al.,[26]). The effect of mercury on the lymphocytes seems to be connected with changes in the level of free calcium in the cytoplasm. The incubation of rat T-cells with methylmercury or with HgCl₂ in the presence of calcium ions demonstrated increased calcium content in the cytoplasm (Tan et al.[27]). Methylmercury increased Ca content rapidly through enhancing its influx through calcium channels and mobilization of Ca²⁺ from the intracellular

Fig. 1 Response surface showing the effects of duration of exposure to Hg⁰ and age of workers on percentage of the peripheral blood lymphocytes.

Fig. 2 Response surface showing the effects of duration of exposure to Hg⁰ and age of workers on percentage of the peripheral blood (CD3+)/T-cells.

Fig. 3 Response surface showing the effects of duration of exposure to Hg⁰ and age of workers on percentage of the peripheral blood (CD4+)/T-helper cells.

Fig. 4 Response surface showing the effects of duration of exposure to Hg⁰ and age of workers on percentage of the peripheral blood (CD8+)/T-suppressor cells.

Fig. 5 Response surface showing the effects of duration of exposure to Hg⁰ and age of workers on percentage of the peripheral blood (CD3+)/T/(CD8+)/T-suppressor ratio.
organelles. In the case of HgCl₂ the increase in Ca²⁺ was much slower because it came only from the incubation medium.

The present study demonstrates that occupational exposure to mercury stimulated the T-cell line but was without any important effect on the count of NK-cells. Only the proportion of NK-cells decreased. Quantitative changes of T-helper cells are thus the immunological indicator of the biological effects of exposure to mercury.

HgCl₂ induces a (CD4⁺)T-cell-dependent systemic autoimmune disease in susceptible strains of rats and mice. In rats, autoreactive T-cells were shown to be involved, whereas in mice, attention has focused on the demonstration of ‘Hg-specific’ T-cells (Kubicka-Muranyi et al.28) Also in mice induction of systemic autoimmunity by mercury was strictly dependent on T-cells, specifically (CD4⁺)T-helper cells (Hultman et al.29) Stimulation of the immune system by metallic Hg was confirmed also by other authors (Bencko et al.,20 Izdebska-Szymona and Kopeć-Szlezak,15 Queiroz et al.,29 White and Brandt.30) The increased counts of lymphocytes, T-cells, T-helper and T-suppressor cells, with normal NK-cell count in peripheral blood were found in dental students but not in medical students (Eedy et al.31) The former had contact with mercury vapours during practical classes, and mercury-containing amalgam is a widely used material in dentistry. Amalgam fillings continue to release microamounts of mercury which can be traced in saliva, blood, urine and even in the exhaled air of individuals having such fillings. Discussion has been continuing for many years to assess whether or not this is a health risk. In two subjects an increase in percentage of T-cells in peripheral blood was noted after removal of amalgam fillings (Eggleston.32) Recently a number of immunological tests were carried out after the insertion of the first amalgam fillings and after removal of such fillings, however, no changes were found in the count of T-cells, helper and suppressor cells and NK-cells in the peripheral blood of these subjects (Wilhelm et al.33)

Conclusion

The quantitative changes in T-cell populations as well as changes in (CD3⁺)/(CD8⁺) T-suppressor ratio and (CD4⁺)/(CD8⁺) T-suppressor ratio in peripheral blood of workers exposed to metallic mercury may represent an immunological indicator of the degree of exposure and may be useful in monitoring the immunotoxicity of mercury.

References