Occupational Exposures to Cd, Ni, and Cr Modulate Titers of Antioxidized DNA Base Autoantibodies

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This study was undertaken to establish whether occupational exposures to derivatives of carcinogenic metals evoke inflammatory immune responses, as determined by the presence of elevated titers of antibodies (Ab) that recognize oxidized DNA bases. Sera obtained from the blood of steel welders (Delavare) and from workers of the Centra Ni-Cd Battery Factory (Poznań, Poland) were analyzed by the enzyme-linked immunosorbent assay. To determine specific and nonspecific binding, an oxidized thymidine—5-hydroxymethyl-2′-deoxyuridine (HMdU)—coupled to bovine serum albumin (HMdU-BSA) as well as mock-coupled BSA (M-BSA) were used as antigens for coating the wells of microtiter plates. Titers of anti-HMdU Ab were significantly elevated in the high Cd and Ni exposure groups (18.3 ± 3.2 vs 10.8 ± 2.1 A405/ml; p<0.05). The sera of the groups with low exposures to Cd and Ni also had enhanced titers of those Ab but those increases were not statistically significant. Interestingly, the Ab titers present in the sera of controls for Cd and Ni exposures appear to be constant regardless of the protein content. In contrast, both lightly and heavily exposed subjects exhibited Ab titers that increased with increasing protein content. When 12 randomly selected workers (4 from each of the control, lightly, and heavily exposed groups) were outfitted with personal monitors, anti-HMdU Ab titers of those workers showed a significant difference between the groups with light (<100 μg/m³) and heavy (>200 μg/m³) exposures to Cd (9.8 ± 3.7 vs 22.1 ± 3.7 A405/ml; p<0.01) and Ni (11.7 ± 1.4 vs 31.0 ± 1.8; p<0.001). Workers exposed to welding fumes exhibited higher anti-HMdU Ab titers than unexposed controls, but the difference was not statistically significant. These results point to anti-HMdU Ab as being potential biomarkers of exposure to proinflammatory and potentially carcinogenic agents. — Environ Health Perspect 102(Suppl 3):221–225 (1994)

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Introduction

Many carcinogenic metal compounds were shown to induce oxidative stress (reviewed in 1–4). These include derivatives of Cr, Ni, and Cd. Even in the absence of serum, the insoluble carcinogenic sulfides of Ni and Cd, as well as CaCrO4, can stimulate human neutrophils, which generate enhanced levels of H2O2 that are comparable to those mediated by the potent tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) (5). Ni and Cd sulfides are known to accumulate in the lungs and are chemotactic for neutrophils (6–9). Having the ability to activate phagocytic cells, those carcinogenic metal derivatives are able to induce oxidative stress (5), a hallmark of inflammation (4). Recently, we showed that Ni3S2 causes an oxidative response in Chinese hamster ovary (CHO) cells as well, which produce substantial amounts of H2O2 in response to incubation with Ni3S2 (10).

Recently, we have shown that people suffering from various types of inflammatory conditions elaborate antibodies (Ab) that recognize and bind to an antigen containing oxidized DNA bases (11–13). Titers of these Ab are significantly higher than those present in the sera of healthy controls. Chronic inflammation induces oxidative stress, which is known to cause oxidation of various cellular macromolecules, including DNA (4). The inflammatory conditions we showed to cause elaboration of high titers of antioxidized DNA base Ab include various types of lupus, psoriasis, and immune complex and neoplastic diseases (11–13). Interestingly, these Ab titers decline in people who are also treated with systemic cytotoxic or anti-inflammatory drugs (12,13). In contrast, treatment of psoriasis with UVB causes an increase in Ab titers (13), which is consistent with UVB having proinflammatory properties.

In those studies, we used the oxidized thymidine 5-hydroxymethyl-2′-deoxyuridine (HMdU) coupled to bovine serum albumin (HMdU-BSA) as an antigen. The Ab that bind to HMdU-BSA were exclusively

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Upon completion of the interview, blood was drawn from a peripheral vein into heparin-containing glass vacutainer tubes. All specimens were labeled using random codes and transferred for initial processing within 3 hr of sampling to the Laboratory at the Institute of Occupational Medicine in Łódź, Poland. Frozen plasma samples were shipped to New York University Medical Center and stored at −80°C until assay.

**Exposure to Welding Fumes Containing Mn, Ni, Cr**

Members of a railroad workers union, who had been full-time professional metal arc welders for at least six months, participated in this study (27 volunteers). These workers either welded rails or structural steel on open railroad tracks or in enclosed shops and utilized a variety of welding electrodes. Frequent use of a high manganese-nickel-chrome electrode in a number of applications was reported by most welders. No recent exposure to stainless steel was reported. No quantitative assessment of the actual exposure to welding fumes was attempted at this time. The 11 volunteer controls were office workers, field railroad supervisors, union representatives, janitors, and laboratory technicians. Data on demographic (age and race), body weight, general health, and smoking status were collected through a questionnaire administered by an interviewer at the time of blood withdrawal. Plasma samples were frozen and stored at −80°C until use.

**Preparation of Antigens**

Antigens, consisting of the riboside of 5-hydroxymethyl uracil (HMU) coupled to bovine serum albumin (HMDU-BSA) and mock-coupled BSA (M-BSA), were prepared as previously described (11–13). Briefly, HMU riboside was coupled to BSA by periodate oxidation followed by borohydride reduction of the product, according to a method developed for coupling normal unoxidized nucleosides (14). Mock coupling was carried out under the same conditions but in the absence of HMU riboside. The crude products were purified of the IgM isotype that are known to arise during acute inflammatory conditions.

The goal of this study was to establish whether people who are occupationally exposed to various potentially carcinogenic metal derivatives also elaborate enhanced levels of Ab that bind to antigens containing oxidized DNA bases.

**Experimental Procedures**

**Study Populations**

**Ni and Cd Exposures.** Male employees of the Centra Ni-Cd Battery Factory located in Poznań, Poland, from five different job categories, were divided into three groups according to their levels of occupational exposure to metals. Individuals who work in the chemistry and panel production departments without any type of body protection are considered heavily exposed to Cd oxide and Ni oxide dusts present at high ambient concentrations (group 1, heavy exposure; 10 subjects). Workers in the assembly and maintenance departments are considered less heavily exposed (group 2, light exposure; 9 subjects). Centra administrative personnel served as a control group (12 subjects).

A brief questionnaire was administered to all subjects to collect data on demographics, height, weight, current smoking habit, number of years worked in their respective departments, and number of hours worked in the 3 days preceding the interview.

![Figure 1. Occupational exposure to Cd and Ni (Poznań, Poland). A scattergram showing distribution of individual mean titer of anti-HMDU Ab within control (○), light exposure (□) and heavy exposure (●) groups.](image)

![Figure 2. Relationship between anti-HMDU Ab titers and their specific activities (A_{app}/μg protein). Controls (+), light exposure (□), and heavy exposure (●) to Cd and Ni.](image)

| Table 1. Monitored exposures to Cd and Ni versus anti-HMDU Ab (Poland)\(^a\). |
|---|---|---|---|---|---|
| Sample | n | Exposure, μg/m³ | Ab, A_{app}/μl | n | Exposure, μg/m³ | Ab, A_{app}/μl |
| Control\(^b\) | 10 | – | 10.8 ± 2.1 | 10 | – | 10.8 ± 2.1 |
| Low\(^c\) | 7 | 20.2 ± 8.6 | 9.8 ± 3.7 | 10 | 49.3 ± 11.9 | 11.7 ± 1.4 |
| High\(^d\) | 5 | 1034 ± 236 | 22.1 ± 3.7* | 2 | 1140 ± 830 | 31.0 ± 1.8*** |

n, number of samples. \(^a\)Paired experiments; \(^*\)p<0.01, \(^**\)p<0.001 by Student’s t-test. \(^b\)Designated as occupational controls due to work assignments away from direct exposure. \(^c\)Monitored exposures.
on a DG-P6 column (Bio-Rad, Melville, NY) and lyophilized.

**Enzyme-linked Immunosorbent Assay**

Assays were carried out in microtiter 96-well plates, which were coated with either HMdU-BSA or M-BSA (10 μg/ml). This allowed determination of specific as well as nonspecific binding on the same plate. In addition to the sample sera and buffer (negative) control, a serum with a known high titer of anti-HMdU Ab was used as a positive control on all of the plates (at least three in HMdU-BSA- and three in M-BSA-coated wells). The presence of a positive control eliminates batch-to-batch variability of antigens and of the goat antihuman secondary Ab. In our previous work we found that anti-HMdU Ab are of the IgM isotype, therefore, we used goat antihuman IgM as the secondary Ab.

Sera were diluted 2.5x10^3 to 1x10^5 times and incubated in the antigen-coated wells at 37°C for 2 hr. Wells were washed three times with PBS containing 0.05% Tween-20, followed by incubation with goat antihuman IgM Ab (not affinity purified; Sigma, St. Louis, MO) labeled with horseradish peroxidase (HRPO) for 1 hr, and washed 3 times. Addition of H_2O_2 and the substrate (o-phenylenediamine) to the wells for HRPO-mediated oxidation during the 0.5-hr incubation allows development of color. The color measured at 492 nm in the microplate reader (Anthos Labtec Instruments, Model 2001, Frederick, MD), is proportional to the amount of antihuman IgM Ab bound to the human serum that interacted with the antigen.

Each serum was analyzed four to eight times at different concentrations and the results are presented as mean values of A_492/μl undiluted serum ± SE. The reproducibility of this assay is 5.4 ± 0.5% (12).

Protein levels were measured in appropriately diluted sera, using bicinchoninic acid (BCA) and Cu^{2+} as reagents, according to the manufacturer’s (Pierce Chemicals, Rockford, IL) conditions. The results of two to four determinations are expressed as mean values ± SE in μg/ml undiluted serum.

**Statistical Evaluation**

The difference between mean values was tested by the Student’s t-test. Correlation coefficients (r) were calculated according to the following formulas:

\[ r = \frac{m \sigma_x}{\sigma_y} = \frac{\bar{Y} \Sigma x - \Sigma x \bar{Y}}{\Sigma x^2 - \Sigma x^2}; \]

\[ b = -m \bar{X} + \bar{Y}; \]

Where m is a slope, b is an intercept, X and Y are mean values of the x and y series (actual exposures to Cd or Ni in μg/m^3 versus Ab titers in A_492/μl in sera of Ni-Cd battery workers), while σx and σy are standard errors of the x and y determinants.

**Results**

**Cd and Ni Exposures**

Figure 1 shows the distribution of anti-HMdU Ab titers in three groups assigned according to occupational exposure to Cd and Ni. Antibody titers of heavily exposed workers are higher than those of the lightly exposed group (18.3 ± 3.2 vs 12.1 ± 3.5 A_492/μl serum), and are significantly (p<0.05) higher than controls (10.8 ± 2.1 A_492/μl serum).

The plot of Ab titers (A_492/μl serum) versus specific activity (A_492/μg protein) shows a high correlation between these two parameters with r = 0.98 (Figure 2). However, the heavily exposed group exhibits a greater increase in Ab titers per unit of proteins present in those sera than do controls and lightly exposed people.

A more detailed analysis (Figure 3) of the relationship between Ab titers (A_492/μl serum) and protein levels (μg protein/μl serum) shows that the control group has a mean Ab value of 10.8 ± 2.1 A_492/μl serum regardless of the protein levels in those samples, which range from 45 to 65 μg/μl, suggesting basically constant anti-HMdU Ab titers. The lightly exposed group shows a linear elevation in the Ab titers with increasing protein content. At the lowest protein level (45 μg/μl), the Ab titer is well

![Figure 3](image_url)

**Figure 3.** Relationship between anti-HMdU Ab titers and protein content (A_492/μl serum vs μg protein/μl serum). Correlation was determined separately in each of the groups, and then combined in one graph. Controls (+), light exposure (○), and heavy exposure (●) to Cd and Ni.
taking antiinflammatory medications, which could have decreased the Ab titers. We found this to be the case in our previous work (12,13). Since medication information was not available for all of the study subjects, we could not take this parameter into account.

Table 2 compares anti-HMdU Ab levels in the sera of workers occupationally exposed to Cd and Ni (Centra battery factory in Poznań, Poland) vs. those exposed to welding fumes (Delaware).

**Discussion**

The results presented demonstrate that occupational exposure to salts of carcinogenic metals induces an inflammatory response, as judged by the elevated titers of autoantibodies that recognize the oxidized DNA base derivative HMdU. Those Ab, which are of the IgM isotype, bind to more than one HMdU residue since free HMdU could not neutralize those Ab, while HMdU-BSA did (7). When workers are divided into groups according to their occupational exposure levels, a significant difference between the controls and those heavily exposed to Cd and Ni is apparent at p<0.05. Actual monitoring of the exposures using personal monitors demonstrates even greater differences, with p<0.01 for Cd and p<0.001 for Ni exposures. These differences are even more remarkable considering that only 12 workers selected at random (four from each occupationally exposed group: controls, light, and heavy exposures) were monitored in this pilot study. These results point to the importance of monitoring actual exposures, particularly since it reduces the possibility of making an error in classification of occupational exposures.

A careful examination of Figure 2 leads one to conclude that although the correlation between $A_{492}/\mu l$ serum and $A_{280}/\mu g$ protein is excellent ($r = 0.98$), the values of the highly exposed subjects deviate in such a way as to show that the rate of increase in anti-HMdU Ab titers is greater than in protein levels (i.e., specific activity is higher than in both controls and lightly exposed subjects).

When the correlation coefficient was determined separately for each of the three groups, the Ab titer did not change in controls regardless of the protein content (Figure 3). In contrast, both low- and high-exposure subjects exhibit Ab titers that increase with the increasing protein amounts. The difference between high-and low-exposure groups is not that much in the slope but rather in the y (Ab titer) intercept. However, the overall increase in specific activity of those Ab appears to be much greater in the case of light exposure to Cd and Ni (9-fold) than of the high exposure (3.6-fold). These results point to the possibility that analysis of changes in specific activities of anti-HMdU Ab provides a more sensitive indicator of occupational exposures. It could be that anti-HMdU Ab titers are constitutive and present at low levels at all times. However, exposure to proinflammatory agents modulates those Ab commensurate with severity of exposure by depleting existing Ab at very low exposures first, and then stimulating their production when increased formation of oxidized bases in DNA occurs.

Comparison of the control groups from the Poznań and Delaware studies (Table 2) points to the importance of determining the distribution of a new biomarker in each of the control populations. It is striking that controls from Delaware have about 2.5 times higher Ab titers (27.4 ± 5.4) than those from the Poznań factory. The Ab titers of healthy controls analyzed in our previous study (12) were 14.9 ± 2.2, as compared to Poznań (10.8 ± 2.1) and Łódź (19.0 ± 2.6) (unpublished data). One possible explanation could be that there are differences in lengths of exposure to the sun and to air pollutants among various population groups, i.e., Łódź. The subjects used as controls for the welders' exposure are likely to spend more time outdoors than the other three populations. We previously have found that UVB exposure significantly enhances the anti-HMdU Ab titers of the patients with psoriasis who were treated with UVB modality (13). Also, a possibility exists that Mn present in the welding fumes counteracts the prooxidant effects of Ni and/or Cr. It was shown previously that Mn dust counteracts Ni-induced toxicity and carcinogenicity (15). Whereas soluble Mn salts inhibit hydroxyl radical-mediated damage in the presence of $H_2O_2$ and superoxide anion radicals (16), conditions present during inflammation and responsible for the oxidation of bases in cellular DNA (4). These considerations could explain why the difference in anti-HMdU titers between control and exposed welder populations is statistically insignificant.

Occupational exposure to Cd and/or Ni was not associated with any of the following: history of smoking, age, weight, and years on the job. However, the small sample does not allow any definite conclusions. Exposure was correlated with anti-oxidized DNA base Ab titers as shown here.
as well as production of autoantibodies that recognize brain glial fibrillary acidic protein (GFAP) and are against neuron-specific, neurofilament proteins (H El-Fawal and H Evans, personal communication). The plans are to compare the results obtained using an assay measuring anti-HMdU Ab with those measuring anti-GFAP and neurofilament protein Ab, to detect subgroups of subjects with a particular antibody pattern, for example high titers of both types of autoantibodies.

The preliminary results presented here combined with the sensitivity of the assay are indicative of the potential usefulness of anti-HMdU Ab titers as biomarkers of exposure to proinflammatory carcinogenic agents.

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