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Immunologic Biomarkers Associated with an Acute Exposure to Exothermic Byproducts of a Ureaformaldehyde Spill

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A community was exposed for several days to formaldehyde (HCHO), hexamethylenetetramine, trimethylamine, and paraformaldehyde emitted from an overheated tanker car containing ureaformaldehyde resin. Residents experienced acute HCHO symptoms at the time of the accident. Many developed chronic, multiple organ health complaints. Three years following the accident, exposed subjects were compared to residents of a nearby unexposed community for the following immunological parameters: white blood cell count, total lymphocyte count, percent and total lymphocyte subsets (CD5, CD4, CD8, CD19, CD25, and CD26 cells), prevelance of autoantibodies, and antibodies to HCHO-human serum albumin (HCHO-HSA) conjugate. The data were adjusted for gender, age, history of smoking, mobile home residency, and use of wood stoves. There was a statistically significant difference for the following: elevated percent and absolute numbers of CD26 cells ($p < 0.0001$); autoantibodies ($p < 0.004$), and greater titers of isotypes IgG ($p < 0.0005$) and IgM ($p < 0.005$) to HCHO-HSA. It is concluded that the exposed subjects had an activated immune system in addition to the elevated autoantibodies. Also, isotypes to HCHO-HSA resulted from the exposure and no other sources, such as smoking, mobile home residency, and use of wood stoves.

Introduction

Chronic, low-level inhalation exposure of humans to formaldehyde (HCHO) is associated with antibodies to HCHO-human serum albumin (HCHO-HSA) conjugate (*1–5*) as well as elevated CD26 cells and autoantibodies (*5*). Other pertubations in immunologic parameters include increased production of histamine (*6*), thromboxane B-2, and PGE-2 (*7*). Commensurate with these findings are health complaints indicative of immunologic sensitivity (*1,6,7*) as well as multiple organ symptoms (*4,5*). Thus, HCHO is an immunogen under chronic, low-level inhalation exposure.

In March 1986, a small community in Alaska was exposed to HCHO and other reactants emitting from an overheated tanker car filled with ureaformaldehyde resin. The Alaska Department of Health and Social Services reported initial symptoms were consistent with exposure to HCHO and reactants (trimethylamine [TMA], paraformaldehyde [PFA], and hexamethylenetetramine [HMTA]). In addition, 50% of those exposed had multiple, recurrent, unresolved health complaints 2 months following the spill (*8*). In this study we present evidence that the lingering health problems are associated with an activated immune system (elevated CD26 cells), autoantibodies, and isotypes to HCHO-HSA conjugate.

Materials and Methods

Tanker Venting and HCHO Concentrations

A railroad tanker car containing 190,000 lb of ureaformaldehyde resin underwent uncontrolled venting in Crown Point, Alaska, March 1986, average ambient temperature of 0°F. The ureaformaldehyde concentrate had the following composition: HCHO (59.6–60.4%), urea (24.5–25.5%), TMA (1%), and methanol (less than 1% maximum). The tanker car was initially steam heated (about 150°F) in anticipation of transport. Approximately 12 hr later the tanker contents were again steam heated for an additional 48 to 49 hr. The temperature of the tanker was measured at 201°F, while interior steam coils attained temperatures exceeding 300°F. Release of ammonia from the urea began an exothermic reaction forming HMTA, carbon dioxide, and water. Approximately 3 days later, the internal pressure increased, and vigorous venting began on March 1. During the next 48 hr, between 85,000 to 99,000 lb of chemical reactants were released. The most likely vented byproducts were carbon dioxide, water, HCHO, HMTA, TMA, PFA, and ammonia. The community was evacuated on day 2 and given permission to return on day 3 following the initial venting. The residents noted a strong "fish odor" attributed to TMA for several weeks following the accident.

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Beginning on March 4, officials from the Environmental Protection Agency (EPA) region 10 and the Alaska Department of Environment Conservation began environmental monitoring in coordination with the Alaska Railroad Corporation. Ambient air and snow samples were collected at approximately 31 different sites in Crown Point. Escaping gas was also sampled. Formaldehyde samples were collected by midget impingers in distilled water at 1 L/min for 1 to 2 hr and analyzed by National Institute of Occupational Safety and Health (NIOSH) P and CAM method 125. Air amine levels were obtained using SKC amine collection tubes #2614, Bendix 44 personal monitor sampler pump and analyzed by NIOSH P and CAM method 221. Snow samples were collected by scraping off the ¼ to ½ inch of snow surface and analyzed by NIOSH P and CAM method 125 (*9*). Ambient concentrations of HCHO during the first 48 hr were estimated with a Pasquill atmospheric stability classification of C (day) and F (night) with a source of 56,000 mg/sec and a source height of 33 feet (*10*). According to these assessments, the citizens of Crown Point were considered exposed to HCHO and reactants emitted from the tanker car.

Exposed and Unexposed Subjects

The exposed subjects consisted of 42 individuals (21 males, 21 females) age 34 ± 16.6 years (range 4–68), who were present at the time of the spill at Crown Point. Subjects were referred for diagnostic testing by their physician. A survey research firm solicited 29 unexposed volunteers (13 males, 16 females) age 54 ± 19 (range 14–80) from the community of Cooper Landing as a comparison group. Cooper Landing is located west and upwind 5 to 10 miles of Crown Point. The plume from the venting moved eastward, never reaching Cooper Landing.

Blood Collection

Blood samples were drawn approximately 3 years following the accident. The samples from both groups were collected and labeled under supervision of an attending physician using silicon-treated, heparinized glass evac-tubes. The blood samples were transported to the laboratory by an overnight carrier and were used within 24 hr following collection. Upon arrival, the specimens were assigned a computer-generated accession number. Quality assurance was performed by positive and negative controls run simultaneously with the unknown samples. Cell viability was 90% by trypan blue exclusion.

HCHO-HSA Conjugation and ELISA Antibody Assay

IgE, IgM, and IgG anti-HCHO-HSA antibodies were determined by an enzyme-linked immunoassay (ELISA) procedure as described elsewhere (*5*), except the conjugate was not frozen.

Lymphocyte Surface Markers

All procedures were performed on heparinized venous blood within 24 hr following collection. The total peripheral white cell (WBC) and lymphocyte counts were performed using a Coulter T 540 counter (Coulter, Florida). Lymphocyte marker procedures are described elsewhere (*2,5*). In brief, peripheral mononuclear cells were isolated by Ficoll Hypaque density gradient (*12*). The percentages and absolute numbers of lymphocte subsets per cubic centimeter of blood were enumerated by fluorescent microscopy using monoclonal antibodies to surface markers as follows: CD5 (LEU1, T-cells), CD5 (LEU3A, T-helper cells), CD8 (LEU2A, T-suppressor cells), CD19 (LEU10, B-cells), (Beckton-Dickinson, Los Angeles, California) and CD25 (IL2+ receptor cells) and CD26 (Tal+ cells) (Coulter, Florida). All surface markers, except CD26, were identified by indirect immunofluorescence (*13*). CD26 cells were determined by a direct immunofluorescent method (*14*).

Autoantibody Screen

Antismooth muscle (ASS), antiparietal cell (APA), antibrush border (ABB), antimitochondrial (AMA), and antinuclear autoantibodies in the subjects' sera were detected by an indirect immunofluorescent method and expressed as positive at a titer of 1:20 (*15*).

Statistical Methods

Two-tailed *t*-tests were performed to determine whether there was a difference between the two groups in the various mean blood parameters, including anti-HCHO-HSA isotypes. The titers for each isotype were converted to geometric means for these analyses. Fischer exact tests, odds ratios, and 95% confidence intervals (CI) were calculated to determine whether the Crown Point residents were at a higher risk for autoantibodies. Correlation analysis was performed to determine if age and gender affected lymphocyte subsets and anti-HCHO-HSA isotypes. Analysis of variance was used to determine whether smoking status and mobile home residency affected HCHO-HSA isotypes and CD26 cells. The Bonferroni inequality was employed.

Results

Formaldehyde Concentrations

HCHO levels in air samples at various sites ranged from 0.007 to 0.093 ppm, with an average of 0.023 ppm on March 5 and 6. Snow level concentrations had a 6-day average of 0.515 ppm and a 1-month average of 0.346 ppm in Crown Point. HCHO concentrations in the vented gases were 17 ppm and 210,000 ppm in tank liquids. Snow values immediately beneath the tank car ranged from 26.5 to 9900 ppm. Background snow levels remote from the spill were < 0.001 ppm.

Estimates of HCHO concentration in the plume ranged from 5 ppm (Crown Point) to 0.1 ppm (about 3 miles southeast) during the first 48 hr. The exposure to Crown Point residents was in excess of 2.0 ppm for this period of time. TMA was noted as detected (detection limit < 0.20 ppm).

Symptoms of Crown Point Residents

The Alaska Department of Health and Social Services reported initial multiple symptoms in Crown Point residents consistent with HCHO exposure. Examples were nasal congestion (70.3%), sore throat (65%), headache (62%), cough (54.9%), conjunctivitis (51.5%), fatigue (51%), rash (47.5%), dizziness

(40.4%), diarrhea (38.6%), shortness of breath (38%), nausea (37%), and nose bleeds (25.7%). Fifty percent had recurrent, unresolved health complaints approximately 60 days following the spill. Identical symptoms for the Cooper Landing group were 34, 25, 10.9, 14.1, 10.9, 20.0, 10.9, 15.6, 9.4, 6.3, and 5.0%, respectively.

Effects of Age, Gender, Wood Stoves, Mobile Homes, and Smoking

Correlation analysis for the effects of age and gender within groups on observed mean values of immune parameters were not significant. The absolute numbers and percent CD26 cells and the geometric mean titers of IgM and IgG isotypes were consistently elevated in male and female Crown Point residents versus their Cooper Landing counterparts.

The use of wood stoves and previous residency in mobile homes did not correlate with antibodies to HCHO-HSA or elevated CD26 cells. For example, of the seven subjects in Crown Point who used wood stoves, three had isotypes to HCHO-HSA and four did not. Only two subjects in Cooper Landing had wood stoves, of which one had IgM (1:8) antibodies to HCHO-HSA.

During the previous 10 years, 10 Cooper Landing residents and 7 Crown Point residents had lived in mobile homes. The duration of occupancy ranged from 2 months to 17 years in (Cooper Landing) and 6 weeks to 6 years (Crown Point). Analysis of variance revealed no difference in mean values of CD26 cells or isotypes of HCHO-HSA. No IgG anti-HCHO-HSA isotypes were found in the Cooper Landing group with respect to either mobile home residency or wood stove usage.

The effect of smoking history on CD26 cells and IgG and IgM isotypes to HCHO-HSA in CP is given in Table 1. *F* values were not statistically significant.

HCHO-HSA Antibodies

The geometric mean titers of IgE, IgM, and IgG isotypes to HCHO-HSA for Crown Point and Cooper Landing subjects are given in Table 2. IgE isotypes were not different in the two groups. IgM and IgG titers were significantly higher in Crown Point group versus Cooper Landing residents, $p < 0.005$ and $p < 0.0005$, respectively.

WBC and Lymphocyte Subsets

The absolute counts for WBC, total lymphocytes, CD5, CD4, and CD8 cells and the CD4/CD8 ratio did not differ in the two groups (Table 3). However, the percentage of CD5 and CD4 cells in the peripheral blood was lower in the Crown Point versus the Cooper Landing residents ($p < 0.05$).

CD19, CD25, and CD26 Cells

No difference between Crown Point and Cooper Landing subjects was found in the absolute and percent CD19 and CD25 cells (Table 4). The CD26 cells were significantly elevated ($p < 0.0001$) in both absolute and percentages in the Crown Point group versus the Cooper Landing residents.

Table 1. Comparison of mean (±SD) values of four blood tests for Crown Point categorized by history of cigarette smoking.[a]

Test	Smoker		Ex-smoker		Nonsmoker		*F*	*p*-value
CD26, number	350.7 (13)	± 425.3	293.2 (6)	± 219.2	246.5 (22)	± 253.7	0.45	NS[b]
CD26, %	10.6 (13)	± 9.5	12.0 (6)	± 11.5	9.6 (22)	± 10.4	0.14	NS
IgG	2.69 (13)	± 0.86	3.33 (6)	± 1.03	2.57 (23)	± 0.95	1.63	NS
IgM	2.62 (13)	± 1.04	3.17 (6)	± 0.98	2.40 (23)	± 0.72	1.92	NS

[a]Total numbers for each category are in parentheses.
[b]NS, nonsignificant.

Table 2. Geometric mean (±SD) titers of IgG, IgM, and IgE isotypes to HCHO-HSA in the exposed and unexposed subjects.

Isotypes	Crown Point ($n = 42$)	Cooper Landing ($n = 27$)[a]	*p*-value
IgE	2.12 ± 0.45	2.00 ± 0.00	NS[b]
IgM	2.57 ± 0.89	2.11 ± 0.42	< 0.005
IgG	2.71 ± 0.95	2.00 ± 0.00	< 0.0005

[a]Two patients did not have titers performed.
[b]NS, nonsignificant.

Table 3. Mean (±SD) absolute numbers of WBCs, lymphocytes, and percent T-cells along with CD4/CD8 ratio found in the peripheral blood in the exposed and unexposed communities.[a]

Cell type	Crown Point ($n = 41$) Absolute numbers, cells/cm³ blood	Cooper Landing ($n = 29$) Absolute numbers, cells/cm³ blood	*p*-value
WBC	6853.66 ± 1543.88	6886.21 ± 1525.84	NS[b]
Lymphocytes	2744.56 ± 892.05	2517.24 ± 550.37	NS
CD5 (%)	1919.10 ± 681.14	1905.69 ± 461.19	NS
	(70.02 ± 10.11)	(75.55 ± 5.57)	(< 0.05)
CD4 (%)	1322.90 ± 466.99	1336.48 ± 323.62	NS
	(48.34 ± 8.58)	(53.07 ± 4.74)	(< 0.05)
CD8 (%)	644.56 ± 251.26	596.55 ± 174.86	NS
	(24.71 ± 7.26)	(23.69 ± 4.53)	NS
CD4/CD8	2.12 ± 0.68	2.24 ± 0.59	NS

[a]Expected ranges: WBC (4,500–10,300); lymphocytes (1,500–4,000); CD5 (8,00–2,530, 65–79%); CD4 (480–1,185, 35–55%); CD8 (220–865, 20–36%); CD4/CD8 (1.65–2.30).
[b]NS, nonsignificant.

Autoantibodies

The frequency of each autoantibody was consistently higher, although not statisitically signficant, in the Crown Point versus the Cooper Landing groups (Table 5). However, when the percent of autoantibodies present in each group was examined, the Crown Point group had a significantly greater odds ratio (95% CI) of having one or more autoantibodies versus the Cooper Landing residents (Fisher's exact test, $p < 0.004$).

Discussion

The residents of Crown Point experienced exposure to HCHO which most likely exceeded the Occupational Safety and Health Administration 8-hr time-weighted average of 1.0 ppm (*16*). For example, HCHO concentrations during the first 48 hr after the

Table 4. The mean (±SD) absolute numbers, percentages, and *t*- and *p*-values obtained for CD19, CD25, and CD26 cells in the exposed and unexposed subjects.[a]

Cell type	Crown Point (n = 41) Absolute numbers, cell/cm³ blood	Cooper Landing (n = 29) Absolute numbers, cells/cm³ blood	*t*-value	*p*-value
CD19 (%)	232.02 ± 244.80	201.93 ± 64.63	0.75	NS[b]
	(7.78 ± 6.73)	(8.03 ± 2.01)	0.22	NS
CD25 (%)	80.39 ± 90.10	61.38 ± 42.42	1.15	NS
	(2.83 ± 3.19)	(2.45 ± 1.66)	0.65	NS
CD26 (%)	286.34 ± 310.29	52.10 ± 43.06	4.77	< 0.0001
	(10.27 ± 10.06)	(2.14 ± 1.83)	5.06	< 0.0001

[a]Expected ranges: CD19 (60–400, 4–15%); CD25 (0–320, 0–8%); CD26 (0–160, 0–4%).
[b]NS, nonsignificant.

Table 5. Summary of the percent of each autoantibody detected in the sera of residents at Crown Point versus those of Cooper Landing.

Autoantibody[a]	Crown Point (n = 42)	Cooper Landing (n = 27)	Odds ratios	95% CI
ASS	35.7	14.8	3.2	10.98, 0.93
APA	16.7	3.7	5.2	44.97,0.60
ABB	14.3	3.7	4.6	39.60, 0.50
AMA	2.4	0.0	—	—
ANA	9.5	0.0	—	—
Number of autoantibodies				
1 or more	50.0	14.8	5.8	19.50, 1.70
2 or more	11.90	3.7	7.1	59.70, 0.84
3 or more	7.1	3.7	2.0	20.20, 0.19

[a]ASS, antismooth muscle; APA, antiparietal cells; ABB, antibrush border; AMA, antimitochondrial; ANA, antinuclear.

spill were estimated to be between 2 to 5 ppm in Crown Point (*10*). In addition, HCHO exposure continued for several more days or even weeks as a result of snow contamination and emissions from other reactants (HMTA, TMA, and PF) (*9*). Thus, it appears most likely that the Crown Point residents sustained continuous, low-level exposure to HCHO and reactants in their homes as well as from their immediate environment. Such exposure conditions would be conducive to continuing health problems such as those described in other environments with low-level HCHO (*5,16*). Moreover, the Alaska Department of Health and Social Services reported recurring symptoms approximately 2 months following the accident (*8*).

Initially we were concerned about various uncontrolled parameters that might affect the tests performed on the peripheral blood of both Crown Point and Cooper Landing residents. These included age, gender, smoking, mobile home residency, and use of wood stoves. Statistical analyses revealed no affect by either age or gender on absolute numbers and percentages of F-cells and their subsets. This confirmed our previous observations (*5*). Moreover, analysis of variance revealed no affect of smoking and mobile home residency on isotypes to HCHO-HSA and CD26 cells. These observations failed to reveal prior sensitization to HCHO in either Crown Point or Cooper Landing residents. Therefore, the data were grouped for further comparisons between the two groups.

The significantly higher titers of IgM and IgG antibodies to HCHO-HSA in the Crown Point group compared to the Cooper Landing residents is indicative of a systemic humoral response to HCHO (Table 2). Similar responses have been demonstrated in other environmental settings (*1,2,5*). In the Crown Point subjects, we were able to show that the anti-HCHO-HSA antibodies were independent of the history of smoking (Table 1), use of wood stoves, and occupancy in mobile homes. The latter was initially surprising because of previous demonstration of a relationship between mobile homes and anti-HCHO-isotypes (*2,5*). However, for the most part, subjects in both Crown Point and Cooper Landing lived in mobile homes for short periods of time (usually weeks) during the past 10 years. Thus, it would appear that their residencies were of insufficient duration to appreciably affect the results obtained on HCHO-HSA isotypes, CD26 cells, and autoantibodies.

Although the total white cell count, lymphocytes and T-cells, and helper/suppressor ratios in the Crown Point residents did not differ from the Cooper Landing group, the Crown Point subjects have evidence of an activated cell mediated immunity (Table 4). First, the CD26 cells are significantly elevated in comparison to the Cooper Landing group ($p < 0.0001$). CD26 expression occurs with antigenic stimulation, and, therefore, is considered antigen memory cells (*14,15,17*). Moreover, circulating CD26 cells and la-positive cells are elevated in various autoimmune disorders (*18–20*). Recently, we demonstrated the elevation of CD26 cells in individuals with chronic health complaints associated with inhalation exposure to HCHO, isocyanates, and chlordane (*5,21,22*). Since an increase in circulating CD26 cells occurs in individuals undergoing chronic stimulation (i.e., chemical sensitivity, autoimmunity), it appears that some of the Crown Point residents in this study have a chronic but subtle activation of the immune system. Recently, it has been suggested that individuals with chronic fatigue syndrome have a chronically activated immune system evidenced by a variety of immunologic abnormalities, including autoantibodies (*23*).

It is recognized that environmental chemicals and therapeutic drugs are associated with autoantibodies, i.e., lupuslike syndrome (*24,25*). The observations made on the Crown Point residents in this study are consistent with chemical exposure and the presence of autoantibodies (Table 5). Also, we have previously demonstrated low titer autoantibodies in individuals exposed to HCHO (*3*) and chlordane (*22*). Moreover, the odds ratio and the Fischer exact tests for the presence ot autoantibodies are statistically significant when Crown Point subjects are compared to the Cooper Landing group. Although the clinical significance of detectable autoantibodies and elevated CD26 is unknown, these immune parameters are associated with the symptoms of the Crown Point residents.

At the present time, autoimmune disorders have not been clinically diagnosed in these patients. However, individuals such as the Crown Point residents with known chemical exposure and subsequent multiple organ symptoms should be examined for autoimmunity. In addition, they should be monitored for any signs of autoimmune problems and subtle alterations in their immune system, i.e., activation. Finally, although we were not part of the original exposure assessment, it is submitted that it is necessary to report these types of observations to make others aware of the necessity for developing better assessment protocols.

In conclusion, measurements of changes in WBC, T-cells, and helper/suppressor ratios in individuals with apparent chemical sensitivities appear to be inadequate immune parameters to examine. If one assumes that these individuals are responding immunologically to environmental chemicals, the investigations

into autoimmunity and immuneactivation as well as pertubations in the interleukins, leukotreins, prostaglandins, and other immunologic mediators appear to be fruitful areas of further research (*5–7,23,25–29*). Thus, it appears that systemic sensitivity to HCHO and probably other toxicants are real phenomena and require further research into the basic components of the immune system.

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