Immune Activation and Autoantibodies in Humans with Long-Term Inhalation Exposure to Formaldehyde

JACK D. THRASHER, Ph.D.
Thrasher & Associates
Northridge, California
ALAN BROUGHTON, M.D., Ph.D.
Antibody Assay Laboratories
Santa Ana, California
ROBERTA MADISON, D.P.H.
Department of Health Science
California State University
Northridge, California

ABSTRACT. Four groups of patients with long-term inhalation exposure to formaldehyde (HCHO) were compared with controls who had short-term periodic exposure to HCHO. The following were determined for all groups: total white cell, lymphocyte, and T cell counts; T helper/suppressor ratios; total Ta1 +, IL2 +, and B cell counts; antibodies to formaldehyde-human serum albumin (HCHO-HSA) conjugate and autoantibodies. When compared with the controls, the patients had significantly higher antibody titers to HCHO-HSA. In addition, significant increases in Ta1 +, IL2 +, and B cells and autoantibodies were observed. Immune activation, autoantibodies, and anti-HCHO-HSA antibodies are associated with long-term formaldehyde inhalation.

INHALATION EXPOSURE to formaldehyde (HCHO) is associated with symptoms of irritation to mucous membranes,1,2 chronic health problems (e.g., asthma,2 nasopharyngeal cancer,3 and multiple subjective health complaints4,5). Recent observations have shown that both humoral- and cell-mediated immunologic mechanisms occur in humans with long-term HCHO exposure. Antibodies of all isotypes to HCHO conjugated to human serum albumin (HCHO-HSA) are demonstrable in HCHO anaphylaxis,6 hemodialysis patients,7 mobile home residents,4 persons with occupational exposures5,8 office workers,9 and in persons in other environments.4 In addition, changes in cell-mediated immunity include increases in eosinophils, basophils, and T-suppressor cells following acute re-exposure of patients with HCHO asthma.10 Moreover, individuals with multiple subjective health complaints associated with long-term HCHO inhalation have evidence of immune activation and the presence of autoantibodies.4,5

The patients in our study had symptoms and complaints related to several organs, as described previously,5,5,9 which were similar to symptoms of workers with multiple chemical sensitivity,11 cacosmia,12 and other chemical exposures.13-15 We report on the differences in humoral and cell-mediated immunity in humans with long-term inhalation exposure to
HCHO vs. asymptomatic students (controls), who experienced short-term, periodic exposure to the chemical.

Materials and methods

Controls and patients. Five groups of subjects exposed to HCHO, who gave informed consent, were included in this study.

(1.) Controls consisted of students of chiropractic medicine (16 males, 12 females, mean age = 29 ± 9 y) exposed to HCHO for 13 h/wk for 28 wk while studying human anatomy. Immunologic tests were performed 12 mo following the last classroom exposure. No measurements of HCHO concentrations were made. It was assumed that classroom ambient concentrations were at least 0.43 ppm. The students stated that during exposure they experienced eye, nose, and throat irritation and that there was a pungent odor of HCHO. They did not have residual health complaints (symptoms), and they were asymptomatic at the time blood was taken.

(2.) Mobile home residents consisted of 19 patients (6 males, 13 females, mean age of 41 ± 20 y) who currently lived in mobile homes. The patients had lived in their environments for 2–7 y and reported multiple symptoms. Measured HCHO concentrations ranged from 0.05 to 0.5 ppm at the time blood samples were drawn.

(3.) Office workers included 21 patients (5 males, 16 females, mean age of 40 ± 10 y) who worked in new office buildings where there was inadequate ventilation (closed buildings). The patients had multiple health complaints. It was determined from medical histories that their symptoms commenced with employment, waned when away from work (i.e., weekends, holidays, vacations) and became worse upon return to work. No HCHO measurements were done; however, closed buildings have ambient concentrations ranging from 0.01 to 0.77 ppm.

(4.) This group included 21 patients (10 males, 11 females, mean age of 35 ± 17 y) who had multiple symptoms and who had been removed from their original sources of HCHO exposure (mobile homes and/or particleboard subflooring) for at least 1 y. The HCHO concentrations measured during their exposures ranged from 0.14 to 0.81 ppm.

(5.) Occupational exposure patients (6 males, 2 females, mean age of 45 ± 11 y) had HCHO exposures from the following: biology and human anatomy classes, mortuary, pathology, physical therapy, formica furniture (particleboard), and carbonless copy paper. Information on six of these patients was reported previously.

Symptoms. All patients in this study had sought continuous medical attention because of multiple organ symptoms involving the central nervous system (CNS) (headache, memory loss, difficulty with completing tasks, dizziness), upper- and lower-respiratory symptoms, skeletal-muscle complaints, and gastroenteritis. Three common symptoms were expressed: (1) an initial flu-like illness from which they had not fully recovered, (2) chronic fatigue, and (3) an olfactory sensitivity to ambient conditions containing low concentrations of chemicals.

One of the students smoked cigarettes (1 pack/d), whereas the remainder and all patients were nonsmokers. No attempt was made to correlate the immunological data with histories of allergies and/or atopy. Previous efforts to make this correlation have led to negative findings.

HCHO-HSA conjugation and ELISA antibody assay. IgE, IgM, and IgG anti-HCHO-HSA antibodies were determined by an ELISA procedure. Conjugation of HCHO with human serum albumin and the ELISA antibody assays were done on sera from freshly drawn blood in accord with information published elsewhere, except the HCHO-HSA conjugate was stored at 4°C.

Lymphocyte surface markers. All procedures were performed on heparinized venous blood within 24 h following collection. The total peripheral white cell count (WBC) was performed using a Model F Coulter Counter (Coulter, FL). The total lymphocyte count was done by blood smear examination. Lymphocyte marker procedures are published elsewhere. In brief, peripheral mononuclear cells were isolated using Ficoll Hypaque density gradient. The percentages and absolute numbers (ABS) of lymphocyte subsets per mm³ blood were determined utilizing monoclonal antibodies to surface markers: LEU1 (T cells), LEU2A (T suppressor cells), LEU3A (T helper cells), LEU10 (B cells) (Beckton-Dickinson, Los Angeles, CA), and Ta1+ and Ta2+ receptor cells (Coulter, FL). All surface markers, except Ta1+, were identified by indirect immunofluorescence. Ta1+ cells were determined by a direct immunofluorescent method.

Autoantibody screen. AntisMOOTH muscle (ASS), antiparietal cell (APC), antibrush border (ABB), antimitochondrial (AMIT), and antinuclear antibodies (ANA) in the subjects’ sera were detected by an indirect immunofluorescent technique and expressed as positive at a dilution of 1:20.

Sex and age effects on cell numbers and autoantibodies. Each of the groups, except occupational, were examined to determine if either sex or age biased the observations on mean absolute counts and percentages of each cell type. Statistical analyses were performed that compared either females with males or younger ages with older ages within each age group. The number of individuals in the occupationally exposed group was insufficient for statistical evaluation for sex and age effects.

Statistical analysis. The student group was used as controls for all statistical tests. Each of the four patient groups were compared with the controls for the following: (a) Z tests were performed to determine whether there was a significantly higher proportion of individuals in each group with antibody titers at or greater than 1:8 to HCHO-HSA; (b) two-tailed t tests and correlation analyses were computed on grouped data to examine any relationship...
between age, gender, WBC, lymphocytes, and lymphocyte subsets in each patient group; and (c) odds ratios and 95% confidence intervals were calculated to determine which groups were at the highest risk of having autoantibodies.

Results

Sex and age effects on cell numbers and autoantibodies. Gender did not affect the mean numbers and percentages of each cell type except as described below. The percentage of Ta1 cells was different in the male office workers \( (p < 0.05) \) because one patient had very high absolute \( (2310 \text{ cells/mm}^3) \) and percentage Ta1 cells \( (44\%) \).

\[ t \] tests revealed no effects of age, but the number of T (LEU1) cells was disparate in controls \( (p < 0.05) \). However, correlations for age effects were not observed \( (r^2 \text{ ranged from 0.00 to 0.42}) \).

Age had no effect on the percentage of autoantibodies. For example, APC (the most common autoantibody) for the younger vs. older individuals was 50% and 60%, respectively (mobile homes), and 89% and 90%, respectively (office workers). The numbers with respect to sex differences were insufficient for evaluation.

As a result of the above observations, all data were pooled regardless of either sex or age for subsequent analyses.

Antibody titers against HCHO-HSA. The antibody titers against HCHO-HSA in the sera of each individual in the five groups are listed in Table 1. The controls had the lowest titers. Titers of 1:16 or greater were predominant in the patient groups. The proportion with positive anti-HCHO-HSA antibodies was least in the controls (39%). The proportion with positive titers was significantly greater \( (p < 0.01) \) for each patient group when compared with the controls.

White blood cells, lymphocytes, T cells, and H/S ratios. The pooled data for WBCs, lymphocytes, T cells, and H/S ratios for each group are summarized in Table 2. The ABS for each cell type and percentages of T (LEU1), T helper (LEU3A), T suppressor (LEU2A), and H/S ratios for all groups fell within the expected reference ranges. We performed \( t \) tests on mean ABS and percentages, using the controls as comparison. Other than the WBCs of the office workers \( (p < 0.05) \), no significant differences from the controls for each patient group were found.

Ta1+, IL2+ receptor, and B cells. The ABS and percentages of Ta1+, IL2+ receptor, and B (LEU10) cells for each group (expected reference ranges are included) are listed in Table 3. The mean values of Ta1+ cells for each patient group as compared with the controls were significantly higher: mobile home residents \( (p < 0.001) \), office workers \( (p < 0.01) \), occupational workers \( (p < 0.01) \), and removed patients \( (p < 0.05) \). The IL2+ cells were elevated in mean ABS and percentages in each of the patient groups \( (p > 0.05) \). The increases were significantly higher in the mobile home residents \( (p < 0.02) \) and the removed patients \( (p < 0.02) \) than in controls. The B (LEU10) cells were also elevated in the four groups vs. controls \( (p < 0.01) \) and they were significantly higher in the office workers \( (p < 0.01) \) and the removed patients \( (p < 0.01) \). The Ta1+ cells exceeded reference ranges in both absolute numbers and percentages in all groups, except the controls. Conversely, both IL2+ and B cells fell within the expected reference values.

Frequency of autoantibodies. The percentage of each autoantibody detected at a dilution of 1:20 in the sera of the subjects in the five groups is listed in

![Table 1](attachment:image.jpg)

*Z and p values are compared with those of controls.

#Two patients did not have IgM titers performed.

July/August 1990 [Vol. 45 (No. 4)] 219
Table 2.—Mean Absolute Numbers of WBCs, Lymphocytes, Percentage T Cells, and H/S Ratios Found in the Peripheral Blood in Each Group

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Controls (n = 27)</th>
<th>Mobile homes (n = 19)</th>
<th>Office workers (n = 21)</th>
<th>Removed (n = 21)</th>
<th>Occup. (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC ABS</td>
<td>6,820 ± 1,740</td>
<td>7,270 ± 2,321</td>
<td>5,900 ± 1,118</td>
<td>6,019 ± 1,173</td>
<td>8,860 ± 4,536</td>
</tr>
<tr>
<td>Lymph. ABS</td>
<td>2,392 ± 707</td>
<td>2,680 ± 737</td>
<td>2,552 ± 914</td>
<td>2,208 ± 599</td>
<td>2,739 ± 1,256</td>
</tr>
<tr>
<td>LEU1 ABS</td>
<td>1,772 ± 576</td>
<td>1,943 ± 648</td>
<td>1,813 ± 664</td>
<td>1,642 ± 607</td>
<td>1,877 ± 718</td>
</tr>
<tr>
<td>(%)</td>
<td>(74 ± 7.7)</td>
<td>(73 ± 13)</td>
<td>(71 ± 12)</td>
<td>(73 ± 10)</td>
<td>(70 ± 8.3)</td>
</tr>
<tr>
<td>LEU3A ABS</td>
<td>1,234 ± 421</td>
<td>1,358 ± 438</td>
<td>1,268 ± 459</td>
<td>1,177 ± 539</td>
<td>1,259 ± 409</td>
</tr>
<tr>
<td>(%)</td>
<td>(49 ± 11)</td>
<td>(51 ± 11)</td>
<td>(50 ± 9.6)</td>
<td>(53 ± 11)</td>
<td>(48 ± 7.5)</td>
</tr>
<tr>
<td>LEU2A ABS</td>
<td>597 ± 219</td>
<td>700 ± 342</td>
<td>682 ± 392</td>
<td>539 ± 205</td>
<td>880 ± 596</td>
</tr>
<tr>
<td>(%)</td>
<td>(25 ± 6.4)</td>
<td>(26 ± 7)</td>
<td>(25 ± 6.9)</td>
<td>(25 ± 7.7)</td>
<td>(30 ± 7.5)</td>
</tr>
<tr>
<td>H/S ABS</td>
<td>2.2 ± 0.72</td>
<td>2.2 ± 1.1</td>
<td>2.1 ± 0.61</td>
<td>2.54 ± 1.3</td>
<td>1.8 ± 0.8</td>
</tr>
</tbody>
</table>

Notes: Absolute numbers are in cells/mm³ of blood. Expected ranges: WBC (4,500–10,300), Lymph. (1,500–4,000), LEU1 (800–2,530, 65–79%), LEU3A (480–1,185, 35–55%), LEU2A (220–865, 20–36%), H/S (1.65–2.3).

Table 3.—Absolute Numbers (ABS), Percentages, and t and p Values Obtained for Ta1 +, IL2 +, and LEU10 (B) Cells in Each Group

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 27)</th>
<th>Mobile homes (n = 19)</th>
<th>Office workers (n = 21)</th>
<th>Removed (n = 21)</th>
<th>Occup. (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ta1 + ABS</td>
<td>122 ± 95</td>
<td>463 ± 306</td>
<td>447 ± 510</td>
<td>236 ± 205</td>
<td>536 ± 290</td>
</tr>
<tr>
<td>(%)</td>
<td>(5.4 ± 3.6)</td>
<td>(20.0 ± 14.7)</td>
<td>(16 ± 12)</td>
<td>(10.8 ± 9.0)</td>
<td>(21 ± 11)</td>
</tr>
<tr>
<td>t</td>
<td>4.713†</td>
<td>2.888†</td>
<td>2.375§</td>
<td>3.73†</td>
<td></td>
</tr>
<tr>
<td>IL2 + ABS</td>
<td>71 ± 45</td>
<td>171 ± 151</td>
<td>107 ± 113</td>
<td>139 ± 113</td>
<td>102 ± 87</td>
</tr>
<tr>
<td>(%)</td>
<td>(3.3 ± 2.7)</td>
<td>(6.2 ± 5.0)</td>
<td>(4.4 ± 3.8)</td>
<td>(6.6 ± 6.1)</td>
<td>(3.4 ± 2.2)</td>
</tr>
<tr>
<td>t</td>
<td>2.801‡</td>
<td>1.377#</td>
<td>2.602‡</td>
<td>0.912#</td>
<td></td>
</tr>
<tr>
<td>LEU10 ABS</td>
<td>143 ± 103</td>
<td>256 ± 278</td>
<td>280 ± 191</td>
<td>310 ± 344</td>
<td>266 ± 282</td>
</tr>
<tr>
<td>(%)</td>
<td>(6.2 ± 4.6)</td>
<td>(8.8 ± 7.3)</td>
<td>(11 ± 7.9)</td>
<td>(13 ± 9.5)</td>
<td>(8.9 ± 4.7)</td>
</tr>
<tr>
<td>t</td>
<td>1.692#</td>
<td>2.969‡</td>
<td>2.151‡</td>
<td>1.319#</td>
<td></td>
</tr>
<tr>
<td>LEU10 (%)</td>
<td>(1.343#)</td>
<td>(2.477)</td>
<td>(2.990)</td>
<td>(1.385)</td>
<td></td>
</tr>
</tbody>
</table>

Notes: Absolute numbers are in cells/mm³ of blood. Expected ranges: Ta1 + (ABS = 0–160, 0–4%), IL2 + (ABS = 0–320, 0–8%), B (LEU10) cells (ABS = 50 to 400, 0–15%).

tp,<.001.
tp,<.01.
tp,<.05.
#Not significant.
#p,<.02.

Table 4. The controls had the lowest frequency of autoantibodies. In contrast, mobile home residents had the highest occurrence of each of the autoantibodies. The most frequently found autoantibody was APC. When the rate at which autoimmunity (i.e., presence of an autoantibody) was determined, the controls had the lowest; the highest rate occurred in the mobile home residents.

Odds ratios for frequency and rate of occurrence of the autoantibodies were performed on the mobile home residents and office workers vs. controls (Table 5). The odds ratio for ASS (8.2) was signifi-
Discussion

Two issues should be addressed before between-group comparisons of the data are made. The first is whether the students suffice as ample controls. The second entails the possible effects of sex and age on the observed differences in anti-HCHO-HSA isotypes and in Tα1 cells and autoantibodies between the controls and the patients.

According to Schlesselman,

controls should be free of the disease being studied. Controls should also be similar to the cases with regard to past potential for exposure. The students met both of these criteria. First, they were asymptomatic. Second, they had similar risks of exposure to HCHO in either the home or office. They did, in fact, have classroom exposure similar to that experienced by the occupational group. The major difference in the exposure between the students and the other patients was one of the duration, i.e., periodic vs. almost continuous, with the exception of the occupational group.

Moreover, despite the intuitive appeal of age and sex matching, there is no equivocal evidence in theory or practice that supports a general preference for this technique. Both t tests and correlation analyses demonstrated that both the mean absolute numbers and percentages of each cell type

<table>
<thead>
<tr>
<th>Table 4.—Autoantibodies: Percentage in Each Group, by Type of Autoantibody and by Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
</tr>
<tr>
<td>---------------------------------------</td>
</tr>
<tr>
<td>ASS</td>
</tr>
<tr>
<td>APC</td>
</tr>
<tr>
<td>ABB</td>
</tr>
<tr>
<td>AMIT</td>
</tr>
<tr>
<td>ANA</td>
</tr>
</tbody>
</table>

Notes: ASS (anti-smooth muscle), APC (anti-parietal cell), ABB (anti-brush border), AMIT (anti-mitochondrial), and ANA (anti-nuclear).

*Numbers are insufficient to perform an odds ratio analysis.
†Rate (percentage) in each group with one, two, three, or four or more autoantibodies.

<table>
<thead>
<tr>
<th>Table 5.—Odds Ratios for the Percentage and Rate of Autoantibodies in Mobile Home Residents and Office Workers as compared to the controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoantibody</td>
</tr>
<tr>
<td>----------------------------------------------------------</td>
</tr>
<tr>
<td>ASS (odds) (95% C.I.)</td>
</tr>
<tr>
<td>APC (odds) (95% C.I.)</td>
</tr>
<tr>
<td>ABB (odds) (95% C.I.)</td>
</tr>
<tr>
<td>AMIT (odds) (95% C.I.)</td>
</tr>
<tr>
<td>ANA (odds) (95% C.I.)</td>
</tr>
<tr>
<td>1 or more (odds) (95% C.I.)</td>
</tr>
<tr>
<td>2 or more (odds) (95% C.I.)</td>
</tr>
<tr>
<td>3 or more (odds) (95% C.I.)</td>
</tr>
</tbody>
</table>

*Confidence intervals are large as a result of small numbers in each group.
†Not calculated because of zero value for control group.
explanation for this difference is simply the lag time (greater) are present in the patients v. controls. One detection. However, the higher titers of IgE and IgM isotypes in the patients suggest that a more recent types of cells support immune activation in the pa-

Although the total white cells, lymphocytes, T cells, and H/S ratios are within expected ranges in the five groups (Table 2), the patients have evidence of an activated cell-mediated immunity (Table 3). First, Ta1+ cells are significantly elevated in the four groups when compared with the controls (p ranges from < .05 to < .001). Ta1+ expression occurs after antigenic stimulation. Also, Ta1+ cells respond to recall antigens and, therefore, are considered antigen memory cells. Moreover, circulating Ta1+ cells and la-positive cells are elevated in various autoimmune disorders. We recently demonstrated elevation of Ta1+ cells in individuals with chronic health complaints associated with HCHO and isocyanate inhalation. Because an increase in circulating Ta1+ cells occurs in individuals undergoing chronic antigenic stimulation (i.e., chemical sensitivity and autoimmunity), the elevation of Ta1+ cells in these patients indicates that they have a chronic immune activation. Furthermore, the disparate numbers for Ta1+ cells of the removed patients in comparison with the controls lend additional support to this conclusion. These patients, along with the others, express an olfactory sensitivity to environmental conditions that elicit symptoms. Thus, higher Ta1+ cells and anti-HCHO isotypes in the removed patients are two immunologic parameters that appear associated with their ongoing health complaints.

Second, both IL2+ and B (LEU10) cells of the four groups of patients show a trend toward elevation as compared with the controls (Table 3). The increase is significant for IL2+ cells in the mobile home residents (p < .01) and removed groups (p < .02). Also, the B cells are increased in the office workers and those removed (p < .05 to < .01). The IL2+ cells occur in acute immune activation and B cells produce antibodies. Therefore, the increases in these two types of cells support immune activation in the patients. The elevation in Ta1+, IL2+, and B cells may result from one or both of the following: (a) immunological memory to, and antibody production against, certain environmental chemicals, and (b) the presence of autoantibodies.

Higher anti-HCHO-HSA isotypes (i.e., 1:16 or greater) are present in the patients v. controls. One explanation for this difference is simply the lag time between the last exposure v. the time of antibody detection. However, the higher titers of IgE and IgM isotypes in the patients suggest that a more recent exposure has occurred, particularly if the higher IgG titers are considered also. In this vein, the patients complain of a sensitivity (both olfactory and respiratory) to environments containing low concentrations of HCHO and other chemicals. Thus, the higher titers may indicate that their immune systems are on constant alert, undergoing continuous activation upon encountering and recognizing environmental haptens. It would be of interest to examine for other haptens to which the patients may be responding.

The higher antibody titers and the larger proportion of individuals with anti-HCHO isotypes in the removed patients v. controls merit comment. Both groups were at least 1 y removed from their original source of exposure. However, the controls were asymptomatic, whereas the patients experienced ongoing health problems associated with environmental exposures, e.g., new carpets, fresh paints, new furnishings, diesel exhaust, and perfumes. Thus, it appears that long-term low-level exposure to HCHO, and possibly other haptens, lead to immunological recognition and immune activation in sensitized individuals. Apparently, shorter periodic exposure to HCHO may lead to recognition but not necessarily immune activation. Moreover, chronic low-level exposures to HCHO appear to effect a sensitivity to environmental chemicals. Perhaps the anti-HCHO-HSA isotypes in these patients is but one aspect of a multiple immunologic response to environmental exposures as observed in building-related illness.

It is recognized that chemicals and therapeutic drugs are associated with a Lupus-like syndrome. The observations made on the patients in this study support this concept. The percentage of specific autoantibodies (e.g., ASS, APC, ANA, etc.) are consistently higher in the patients v. controls (Table 4). Moreover, the odds ratios for the presence of at least 1, 2, or 3 autoantibodies are significantly greater in the residents of mobile homes and office workers (p < .05) relative to controls (Table 5).

Presently, autoimmune disorders have not been diagnosed clinically in these patients. However, current investigations in progress appear to correlate the presence of APC autoantibodies with gastrointestinal complaints and antemyelin autoantibodies with CNS and PNS symptoms.

In conclusion, measurements of changes in WBCs, T cells, and H/S ratios in individuals with apparent chemical sensitivities appear to be inadequate immune parameters to examine. If one assumes that these individuals respond immunologically to environmental chemicals, investigations into autoimmunity and immune activation and perturbations in the interleukins, leukotrienes, prostaglandins, and other immunologic mediators appear to be fruitful areas for further research. Thus, it appears that HCHO sensitivity is a real phenomenon and requires further research.
We wish to thank Drs. Heuser and Baker for referring some of the patients in this study. Valuable technical assistance was obtained from Mr. Gilbert Salizar and the technical staff. Submitted for publication November 15, 1989; revised; accepted for publication March 13, 1990.

Requests for reprints should be sent to Jack D. Thrasher, Ph.D., Thrasher & Associates, 11330 Quail Creek Rd., Northridge, CA 91326.

References