

1987 42:347-50

Evidence for Formaldehyde Antibodies and Altered Cellular Immunity in Subjects Exposed to Formaldehyde in Mobile Homes

JACK D. THRASHER, Ph.D.
Thrasher & Associates
11330 Quail Creek Rd.
Northridge, California
ARISTO WOJDANI, Ph.D.
GEOFFRY CHEUNG, Ph.D.
Allergy Immuno Technologies, Inc.
1527 Monrovia Blvd.
Newport Beach, California
GUNNAR HEUSER, M.D., Ph.D.
Cedars-Sinai Medical Tower
8631 W. Third St., STE 1025E
Los Angeles, California

ABSTRACT: Eight symptomatic individuals chronically exposed to indoor formaldehyde (HCHO) at low concentrations (0.07–0.55 ppm) were compared to 8 nonexposed subjects with respect to: (1) presence of IgG and IgE antibodies to HCHO conjugated to human serum albumin (F-HSA); (2) the percentage of venous blood T and B cells by E and EAC-rosetting; and (3) the ability of T and B cells to undergo mitogen (PHA, PWM) stimulated blastogenesis as measured by the incorporation of tritiated thymidine. Anti-F-HSA IgG, but no IgE, antibodies were detected in the sera of the 8 exposed subjects; none were found in 7 of the unexposed controls. T lymphocytes were decreased in the exposed ($48 \pm 11.5\%$) compared to the control ($65.9 \pm 4.97\%$) subjects ($p > .001 < .01$). B cells were $12.6 \pm 1.6\%$ (HCHO group) and $14.75 \pm 2.1\%$ (controls) ($p > .02 < .05$). The incorporation of labeled thymidine by T cells (PHA) was decreased: $17,882 \pm 2,293$ cpm (HCHO group) and $28,576 \pm 3,807$ cpm ($p > .001 < .01$). T and B cell blastogenesis (PWM) was $9,698 \pm 1,441$ cpm (HCHO group) and $11,279 \pm 1,711$ (controls) ($p > .05 < .1$). Exposure to HCHO appears to stimulate IgG antibodies to F-HSA and decrease the proportion of peripheral T cells.

CHRONIC EXPOSURE to low concentrations of formaldehyde (HCHO) in indoor air is common in occupational and nonoccupational environments.¹⁻³ These individuals have many health complaints that involve the skin, eyes, upper and lower respiratory tract, central nervous system, and the gastrointestinal tract.¹⁻⁶ Symptoms

suggest systemic involvement and may reflect that HCHO acts as a hapten to produce antibodies.⁷⁻¹²

Individuals chronically exposed to low concentrations of indoor HCHO have fatigue, headache, vertigo, and upper and lower respiratory irritation that are difficult to classify by a single mechanism.^{2,7} Early observa-

Table 1.—Anti-F-HSA IgG Antibody Titers as Determined by the ELISA Procedure

Mobile home occupants	Control subjects	HSA coated wells
1:8	1:4	1:4
1:64	1:8	1:4
1:32	1:4	1:4
1:128	1:4	1:4
1:16	1:4	1:4
1:32	1:4	1:4
1:64	1:4	1:4
1:256	1:4	1:4

and were 1:8 in the subject with Parkinson's disease. No antibodies (IgE or IgG) were detected in the micro ELISA wells coated with HSA. Thus, cross reactivity in the assay for IgG antibodies was absent.

T and B cell enumeration. T lymphocytes were decreased in the exposed vs. the control subjects (Table 2). In the HCHO-exposed individuals, T cells ranged from 34% to 65%, mean $48 \pm 11.5\%$ of the mononuclear cells. Controls ranged from 59% to 65%, mean $65.9 \pm 4.97\%$. The difference between means was significant, $p > .001 < .01$.

B lymphocytes in the HCHO-exposed individuals ranged from 11–15%, mean $12.6 \pm 1.6\%$. Controls ranged from 12–18%, mean $14.75 \pm 2.1\%$. The difference between the means was not significant, $p > .02 < .05$.

T and B cell blastogenesis. T cell blastogenesis, as determined by PHA stimulation, was lower in the exposed group than in the controls (Table 2). The uptake of 3H-Tdr in the HCHO-exposed subjects averaged $17,882 \pm 2,293$ cpm, while that for the controls was $28,576 \pm 3,807$. The difference between the means was significant, $p > .001 < .01$.

T and B cell blastogenesis, as determined by PWM stimulation, appeared lower in the experimental group vs. the controls (Table 2). The uptake of 3H-Tdr averaged $9,698 \pm 1,441$ cpm in the HCHO-exposed individuals, and controls were $11,279 \pm 1,711$ cpm. The difference between the means was significant, $p > .05 < .1$.

Discussion

Inhaled HCHO is incorporated into nucleic acids or is covalently bound to proteins in the nasal mucosa of rats.¹⁴ Inhalation exposure causes nasal epithelial cell proliferation and metaplasia, congestion, and secretion in rodents and monkeys.^{20–22} Human subjects experience rhinitis, congestion, and epistaxis following chronic HCHO exposure in the homes.^{2,4–6} Thus, damage to mucosal tissue, including capillary beds, could facilitate HCHO conjugation with blood proteins. IgG antibodies found in this study appear to be directed towards F-HSA conjugates. This is deduced because antigen/antibody cross reactivity to HSA was not observed and IgE and IgG antibodies to F-HSA were absent or minimal in the asymptomatic controls. Thus, anti-F-HSA antibodies probably indicates exposure to HCHO. IgG subtype 4 antibodies are under investigation in individuals exposed to airborne HCHO.

IgE antibodies may have escaped detection because they are present in trace amounts and have a half-life of approximately 2 days.²³ At the time of this investigation these facts were not considered. Subsequently, IgE anti-F-HSA antibodies have been detected.²⁴

Modulation of cell-mediated immunity (CMI) by HCHO has been demonstrated in rodents. Mice exposed for 21 days (6 hr/day, 15 ppm) showed enhanced macrophage H_2O_2 production. In addition, resistance to *L. monocytogenes* challenge was significantly increased.²⁵ Conversely, F-344 rats in a 4-wk study (HCHO for 6 hr/day at 3, 16, or 32 ppm) had depressed numbers of peripheral B cells at the higher concentrations and decreased production of antibodies to sheep RBCs.²⁶ In this study, the exposed subjects had a decreased percentage and blastogenesis of T cells when compared to the controls. Similar observations have been reported in humans exposed to a variety of environmental agents.²⁷ Although the mechanisms responsible for changes in the CMI are poorly understood, they may be related to chronic antigenic stimulation by F-HSA.^{24,28} Also, PWM stimulates both B and T cells, while PHA induces mitosis in T cells. Thus, the differences between the response of T and B cells of these subjects is interpreted as T cell rather than B cell modulation of the CMI. In addition, B cell modulation may be minimal in humans.^{24,28}

Table 2.—Summary of the Observations and Statistics on T- and B-Cell Numbers and Blastogenesis in HCHO-Exposed and Control Subjects

Observation	Mobile home occupants	Control subjects	p value (t test)
T-cell number	$48 \pm 11.5\%$	$65.9 \pm 4.97\%$	$> .001 < .01$
B-cell number	$12.6 \pm 1.6\%$	$14.75 \pm 2.1\%$	$> .02 < .05$
T-cell (PHA) blastogenesis	$17,882 \pm 2,293$ cpm	$28,576 \pm 3,807$ cpm	$> .001 < .01$
T/B-cell (PWM) blastogenesis	$9,698 \pm 1,441$ cpm	$11,279 \pm 1,711$ cpm	$> .05 < .1$

tions, however, indicate that HCHO is a hapten, binding to human serum albumin (HSA) to form a new antigenic determinant, F-HSA.⁸ Recently, IgE antibodies to F-HSA have been demonstrated in anaphylactic shock caused by HCHO in long-term hemodialysis.⁹ Also, IgG and IgM hemolytic anti-HCHO red blood cell (RBC) antibodies are present under similar hemodialysis conditions.^{10,11} Moreover, an anti-F-HSA IgG has been produced in dogs by the intravenous administration of HCHO and F-DSEA.¹² More recently, IgG antibodies to F-HSA have been found in asymptomatic renal dialysis patients, IgE antibodies in two nurses with a history of HCHO asthma, and no antibodies in two physicians with HCHO rhinitis.¹³ Airborne HCHO apparently conjugates with proteins of the upper respiratory tract.¹⁴ This study examined for antibodies to F-HSA in subjects chronically exposed to low concentrations of HCHO in indoor air. In addition, the numbers and blastogenesis of peripheral T and B lymphocytes were studied.

Materials and methods

HCHO-exposed subjects. Seven of the subjects resided in mobile homes. One worked in the laboratory and lived in a newly decorated energy-efficient apartment. They had multiple health complaints¹⁻⁶ recorded on a symptom checklist in their home developed by Godish.¹⁵ A medical history and physical examination showed that three subjects had a history of allergies, but otherwise no obvious explanation for their health complaints. Physical examinations and diagnostic tests (complete blood count, urinalysis, SMAC-24) were normal. One male who was 63 yr of age smoked 20 cigarettes per day, while one female, age 28, smoked 0-5 cigarettes per day. The subjects had lived 2-7 yr in their current residences. The exposed subjects included five females who were 28, 55, 58, 65, and 75 yr of age and three males who were 63, 70, and 70 yr of age. They comprised three married couples and two single females.

Unexposed (control) subjects. The control subjects consisted of four laboratory personnel and four elderly patients. The laboratory personnel included three men, ages 42, 47, and 35 yr, and a 35-yr-old woman, all of whom were asymptomatic. One (age 47) smoked two packs of cigarettes per day. The four elderly patients (i.e., two couples) were being treated for whiplash (male, 75); probable stroke (male, 75); migraine headaches and multiple pollen and grass allergies (female, 63); and Parkinson's disease (female, 67).

HCHO measurements. HCHO was measured by the County Health Departments in Fresno (two mobile homes) and Los Angeles (two mobile homes) counties, utilizing the Na bisulfite impingement method recommended by the Occupational Safety and Health Administration (OSHA). Three of the homes had two separate measurements, while the third had one HCHO measurement. The concentrations of HCHO in ppm and yr of occupancy were, respectively: 0.07, 0.16 (6 yr); 0.110, 0.117 (6 yr); 0.33, 0.33 (7 yr); and 0.55 (7 yr). Formaldehyde measurements were not done in the laboratory worker's apartment nor in the homes of the control subjects, which were at least 20 yr

old. It was assumed that HCHO concentrations would be similar to those reported and estimated by the Environmental Protection Agency,¹⁶ i.e., a mean value of 0.03 ppm.

HCHO antibodies. Formaldehyde (F) was conjugated to chromatographically pure human serum albumin (Sigma) following the procedures of Patterson et al.^{12,13} Lymphocytes were separated from venous blood of exposed and control subjects and immediately used for T and B cell enumeration and blastogenesis. The sera were used to measure IgG and IgE antibodies to F-HSA by the methods of Patterson et al.^{12,13}

The anti-F-HSA antibodies were determined by the ELISA procedure.¹⁷ In brief, micro ELISA plates (Dynatech Laboratories, Alexandria, VA) were coated with various dilutions of either F-HSA or HSA as antigen sources. After incubation for 2 hr, the plates were washed with PBS-Tween (0.5%, Sigma). Dilutions of test and control sera were made in PBS-Tween and incubated for 2 hr. Diluted sera (200 μ l) added to individual wells and the plates incubated for 2 hr. The plates were then washed three times with PBS-Tween and 200 μ l of alkaline phosphatase labeled anti-human IgG or anti-human IgE were added. The plates were then incubated for 12 hr at 4°C, washed in PBS-Tween, and 200 μ l of substrate (paranitrophenyl phosphate) was added. Thirty minutes later the reaction was stopped by adding 50 μ l of 3 mol/L of sodium hydroxide. The optical densities were read at 405 nm on an automatic ELISA reader (Dynatech Laboratories, Alexandria, VA). ELISA end-point titers were read when the optical density reading of the test sera (HCHO exposed) was 2 times that of the negative control (HSA) wells.

T and B enumeration and blastogenesis. T and B cells were enumerated by E- and EAC-rosetting methods, respectively.¹⁸ In both methods, lymphocytes with binding of more than two sheep RBCs were considered positive. The blastogenesis of T and B cells was measured by mitogen activation using phytohemagglutinin (T cells) and pokeweed mitogen (T and B cells). The lymphocytes from both exposed and control subjects were cultured at 37°C in an atmosphere of 95% air and 5% carbon dioxide. The cultures were done in quadruplicate in wells of flat bottom micro-titer plates with optimum concentrations of either PHA (4.0 μ g/ml) or PWM (8.0 μ g/ml). After 48 hr the cells were labeled with 2 μ Ci of tritiated thymidine per well for 24 hr. The lymphocytes were harvested and the incorporation of the labeled thymidine into cellular DNA was measured by liquid scintillation counting. The data were expressed as cpm \pm standard deviation for each of the exposed and nonexposed subjects. The variability in the blastogenesis assay was less than 20%.¹⁹

Results

F-HSA antibodies. IgE antibodies to F-HSA were not detected in exposed and unexposed subjects (Table 1). IgG antibodies to F-HSA ranged from 1:8 to 1:256 in the mobile home occupants. Anti-F-HSA antibodies were not detected in seven of the control subjects (titers 1:4)

Submitted for publication June 15, 1987; revised; accepted for publication July 2, 1987.

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

REFERENCES

- Bernstein, R.S.; Stayner, L. T.; Elliot, L.J.; Kimbrough, R. K.; and Blade, L. 1984. Inhalation exposure to formaldehyde: An overview of its toxicity, epidemiology, monitoring and control. *Am Ind Hyg Assoc J* 45: 778-85.
- Guptka, K. 1984. *Health Effects of Formaldehyde*. Washington, DC: US Consumer Product Safety Commission.
- National Institute of Occupational Safety and Health. 1976. *Occupational Exposure to Formaldehyde*, NIOSH criteria for a recommendation standard. Washington, DC: U.S. Government Printing Office.
- Breyse, P.A. 1979. *Formaldehyde Exposure in Mobile and Conventional Homes*. Proceedings of 43rd Annual Education Conference, National Environmental Health Association.
- Dally, K.A.; Hanrahan, L.P.; and Woodbury, M. A. 1981. Formaldehyde exposure in nonoccupational environments. *Arch Environ Health* 36: 277-84.
- Gamble, J. 1983. *Formaldehyde Toxicity*, pp. 175-97. Washington, DC: Hemisphere Publishing Corp.
- Imbus, H. R. 1985. Clinical evaluation of patients with complaints related to formaldehyde exposure. *J Allergy Clin Immunol* 76: 831-40.
- Horsfall, F.L. 1934. Formaldehyde hypersensitivity: An experimental study. *J Immunol* 27:569-81.
- Maurice, F.; Rivory, J.-H.; Larsson, P.H.; Johansson, S.G.O.; and Bousquet, J. 1986. Anaphylactic shock caused by formaldehyde in a patient undergoing long-term hemodialysis. *J Allergy Clin Immunol* 77: 594-97.
- Fassbinder, W.; Pilar, J.; Scheurmann, E.; and Koch, K.M. 1978. Formaldehyde in the formation of haemodialysis N-like antibodies. *Vox Sang* 39: 41-48.
- Lynen, R.; Rothe, M.; and Gallasch, E. 1983. Characterization of formaldehyde-related antibodies encountered in hemodialysis patients at different stages of immunization. *Vox Sang* 44: 81-89.
- Patterson, R.; Harris, K.E.; and Grammer, L.C. 1985. Canine antibodies against formaldehyde-dog serum albumin conjugates. *J Lab Clin Med* 106: 93-100.
- Patterson, R.; Pateras, V.; Grammer, L.C.; and Harris, K.E. 1986. Human antibodies against formaldehyde-human serum albumin conjugates or human serum albumin in individuals exposed to formaldehyde. *Int Archs Allergy Appl Immun* 79: 53-59.
- Casanova-Schmitz, M.; Starr, T.B.; and Heck, d.'A. 1984. Differentiation between metabolic incorporation and covalent binding in the labeling of macromolecules in the rat nasal mucosa and bone marrow by inhaled [^{14}C] and [^3H] formaldehyde. *Toxicol Appl Pharm* 76: 26-44.
- Godish, T. 1983. *Formaldehyde—A Physician's Guide*. Department Natural Resources, Ball State University, No. 7.
- U.S. Environmental Protection Agency. 1976. *Investigation of Selected Potential Environmental Contaminants: Formaldehyde*, Publ. No. 560/2-76-009. Washington, DC: U.S. Government Printing Office.
- Voller, A. 1979. Heterogeneous enzyme-immunoassays and their applications. In *Enzyme Immunoassay*, E.T. Maggio, ed., pp. 181-96. Boca Rotan, FL: CRC Press.
- Garvey, J.S.; Cremer, N.E.; and Sussdorf, D.H. 1977. *Methods in Immunology*, p. 443. Reading: Benjamin/Cummings.
- Wojdani, A. and Alfred, L.J. 1983. In vitro effects of polycyclic aromatic hydrocarbons on mitogen activation of mouse T-lymphocytes: Action of histamine. *Cell Immunol* 77: 132-38.
- Swenberg, J.A.; Gross, E.A.; and Martin, J. 1982. *Formaldehyde Toxicity*, pp. 132-47. Washington, DC: Hemisphere Publishing Corp.
- Swenberg, J.A.; Gross, E.A.; Randall, H.W.; and Barrow, C.S. 1983. *Formaldehyde*, pp. 225-36. New York: Dekker.
- Rusch, G.M.; Clary, J.J.; Rinehart, W.E.; and Bolte, H.F. 1983. A 26-week inhalation toxicity study with formaldehyde in the monkey, rat, and hamster. *Toxicol Appl Pharm* 68: 329-43.
- Hood, L.E.; Weissman, I.L.; Wood, W.B.; and Wilson, J.H. 1984. *Immunology*, pp. 436-65. Menlo Park: Benjamin/Cummings.
- Broughton, A. and Thrasher, J.D. 1988. Humoral and cell mediated immunity in individuals chronically exposed to formaldehyde. *Comments on Toxicology* (in preparation).
- Dean, J.H.; Lauer, L.D.; House, R.V.; Murray, M.J.; Stillman, W.S.; Irons, R.D.; Steinhagen, W.H.; Phelps, M.C.; and Adams, D.O. 1984. Studies of immune function and host resistance in B6C3F mice exposed to formaldehyde. *Toxicol Appl Pharmacol* 72: 519-29.
- Leach, C.L.; Sharma, R.P.; and Oberg, S.G. 1986. The effects of formaldehyde on the rat immune system (submitted for publication).
- Dean, J.H.; Luster, M.I.; Munson, A.E.; and Amos, H., Eds. 1985. *Immunotoxicology and Immunopharmacology*. NY: Raven Press.
- Thrasher, J.D.; Broughton, A.; Micevich, P.; and Gard, Z. Immunological evaluation of four individuals occupationally exposed to formaldehyde: Antibodies and Immune Profiles (in preparation).