

Evaluation of a worker with possible formaldehyde-induced asthma

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Background: We describe the evaluation of a worker with clinical symptoms compatible with bronchospasm caused by formaldehyde exposure.

Methods: The worker was evaluated by means of enzyme-linked immunosorbent assay, cutaneous tests, and methacholine and formaldehyde inhalation challenges. The worker's serum was injected intradermally into the skin of a normal rhesus monkey to determine whether hypersensitivity could be transferred from human to primate.

Results: An enzyme-linked immunosorbent assay showed that the worker had positive IgE and IgG titers to formaldehyde-human serum albumin. The worker had a positive cutaneous test for formaldehyde-human serum albumin, and this cutaneous reactivity was transferred to a rhesus monkey through the worker's serum. The worker had a negative methacholine challenge at 25 mg/ml and negative formaldehyde inhalation challenges at 0.3, 1, 3, and 5 ppm for 20 minutes. It is possible that the worker would have had a positive result if a higher concentration of F were used for the challenge, but it is more probable that the worker's symptoms were not caused by immunologically mediated asthma. We have studied individuals exposed to formaldehyde, their clinical syndromes, and serologic results for a decade. This worker is the one subject with the most compatible history and immunology, but the worker had a negative challenge.

Conclusion: Immunologically mediated asthma caused by formaldehyde is extremely rare, if it exists at all. (*J ALLERGY CLIN IMMUNOL* 1993;92:29-33.)

Key words: Formaldehyde, asthma, antibody, IgG, IgE, inhalation challenge

There are numerous reports of immunologic and respiratory responses that may occur as a result of the inhalation of reactive, low molecular weight chemicals.¹ As a model of such chemicals, trimellitic anhydride is known to cause three immunologically mediated respiratory syndromes: asthma/rhinitis, late respiratory systemic syndrome, and the pulmonary disease-anemia syndrome.² In contrast to results of exposure to trimellitic anhydride, the pulmonary responses and immunologic correlates of exposure to gaseous formaldehyde are not well defined.

Abbreviations used

F-HSA:	Formaldehyde-human serum albumin
HSA:	Human serum albumin
ELISA:	Enzyme-linked immunosorbent assay
FEV ₁ :	Forced expiratory volume in 1 second

Exposure to formaldehyde is ubiquitous in both occupational and nonoccupational settings. There have been reports of positive challenges³⁻⁵ and negative challenges⁶ in individuals who have symptoms compatible with formaldehyde-induced asthma. None of the reports included immunologic evaluation of specific IgE against formaldehyde or formaldehyde-protein conjugates.

We have been studying immunologic and respiratory responses to formaldehyde in clinical and animal models for the past decade. In dogs immunized intravenously with formaldehyde, we have reported the transient development of immediate-type hypersensitivity.⁷ In human beings undergoing hemodialysis with formaldehyde-ster-

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ilized dialyzers, we reported antibodies against formaldehyde-human serum albumin (F-HSA) of four isotypes: IgG, IgA, IgM, and IgE. In those patients there was no disease associated with the presence of antibody; that is, the patients with IgE to F-HSA did not experience anaphylaxis, nor did the patients with antibody of other isotypes experience symptoms compatible with immune complex disease.⁸ We have reported three other series of individuals exposed to formaldehyde in whom we were unable to associate an immunologically mediated disease with the presence of antibody against F-HSA: individuals exposed to formaldehyde for whom physicians requested antibody determinations⁹; 37 workers exposed to formaldehyde¹⁰; and 55 individuals including 10 histology technicians, 10 pathology residents, 10 medical residents, 10 smokers, 5 miscellaneous workers exposed to formaldehyde and 10 normal subjects.¹¹ In short, it is not established that formaldehyde inhalation causes any immunologically mediated disease.¹²

This report describes our evaluation of a worker with symptoms compatible with bronchospasm caused by formaldehyde exposure and with compatible immunologic findings: positive skin test results to F-HSA and positive IgE against F-HSA.

METHODS

Preparation of F-HSA

The method of F-HSA preparation has been previously described.⁷ Human serum albumin (HSA) (25% solution, American Red Cross Blood Services, Washington, D.C.) was exposed for 30 minutes at 37° C to formaldehyde (Fisher Scientific, Fairlawn, N.J.) (mg/mg). The F-HSA was dialyzed extensively against phosphate-buffered saline and was sterilized with a 0.2 μ m filter (Millipore Corp., Bedford, Mass.). To determine the extent of conjugation, immunoelectrophoretic¹³ and free amino group analytic¹⁴ methods were used.

Enzyme-linked immunosorbent assay method for detection of IgE and IgG to F-HSA and HSA

The enzyme-linked immunosorbent assay (ELISA) method was performed with modifications of previously described methods.^{15, 16} Micro-ELISA Immulon plates (Greiner and Sons, Nürtingen, West Germany) were coated sequentially with each of the following: F-HSA or HSA, dilutions of the worker's serum or control sera, rabbit anti-human IgG or IgE, alkaline phosphatase-conjugated goat anti-rabbit IgG, and p-nitrophenyl phosphate. Appropriate washes with phosphate-buffered saline-Tween were performed between coatings, and the optical density of each well was read at 405 nm.

A serum sample was considered to be positive if its optical density was at least twice that of the negative control. The titer was the last positive dilution. Serial samples were obtained over a 4-year period.

Cutaneous tests for F-HSA, HSA, and usual aeroallergens

Assessment of cutaneous reactivity was determined by standard prick and intradermal techniques¹⁷ with F-HSA, HSA, and usual aeroallergens (elm, oak, ryegrass, Bermuda grass, ragweed, *Dematiaceae* and *Aspergillus* species, house dust mite, cat, dog) (Allergy Laboratories of Ohio, Columbus, Ohio). The criterion for a positive test result was a 5 mm wheal with surrounding erythema.

Methacholine inhalation challenge

Methacholine was administered via a French-Rosenthal inhaler in graded doses; the final dose was 25 mg/ml. A drop of $\geq 20\%$ in forced expiratory volume in 1 second (FEV₁) was the criterion for a positive challenge.¹⁸

Formaldehyde delivery

The effluent from a permeation device (VICI Metronics, Santa Clara, Calif.) was mixed with an airflow of 9 to 12 L/min in a reservoir bag. The permeation device was submerged in an oil bath at carefully regulated temperatures. Airflow and the permeation tube temperature were adjusted to achieve the desired inspired formaldehyde concentrations. Actual values were verified with an AirScan chemical monitor (Crystal Diagnostics, Inc., Wolburn, Mass.).

Formaldehyde inhalation challenge

A single-blind formaldehyde inhalation challenge was conducted according to the method of Frigas et al.⁶ One modification was an additional challenge of 5 ppm for 20 minutes, which was administered after we determined that all lower concentrations were negative. A drop of $\geq 20\%$ in FEV₁ was the criterion for a positive challenge.

Subject

The subject was a 52-year-old man in good health who had the onset of dyspnea, chest tightness, and cough. He also had sneezing and rhinorrhea. These started several months after he began working in a formaldehyde manufacturing plant. His symptoms did not occur during usual daily operations but only immediately after exposure to higher concentrations, such as when a pipe would leak or a machine would break down, necessitating repair and higher exposure levels. When symptoms occurred, he left the area and used a bronchodilator; his symptoms subsided within an hour. The symptoms had been occurring for 5 years at the time of initial evaluation. He was not challenged until 3 years later at which time he practiced avoidance mea-

tures, such as respirator-protection, and had minimal symptoms. He had no symptoms for several months. No other exposures caused similar symptoms. He did not smoke. His only medication was Lopid 600 mg twice daily for treatment of hypercholesterolemia.

His chest x-ray film revealed calcification in hila, slight increase in markings, and blunting of the left costophrenic angle, all of which had been present on previous films. Results of electrocardiogram, complete blood cell count, urinalysis, and SMA20 were normal except for a cholesterol level of 230 mg/dl.

This study was approved by the Northwestern University Institutional Review Board and the Northwestern Memorial Hospital Office of Research, and informed consent was obtained.

RESULTS

Industrial hygiene data

Periodic personal sampling for formaldehyde had been performed during routine operations by the company for several years. The time-weighted average ranged from 0 to 0.2 ppm for inside operators and 0 to 0.45 ppm for outside operators. The 15-minute sampling peak exposures were 0.6 to 0.7 ppm for inside operators and 1.5 ppm for outside operators. This employee worked in both locations.

Immunologic evaluation

The worker had a positive prick test result to F-HSA (10 mg/ml), histamine (1 mg/ml), and house dust mite (10,000 AU/ml). All other test results were negative. As determined by intradermal titration, the worker had a positive reaction to 0.1 µg/ml F-HSA and a negative reaction to HSA. The worker's serum was injected intradermally into the skin of a normal rhesus monkey. Forty-eight hours later, the skin test site was challenged with an intradermal injection of F-HSA, after a nonirritating dose was determined. A positive immediate-type reaction occurred. The result for the control site injected with F-HSA was negative. This test demonstrated a human-to-primate transfer of IgE-mediated immediate-type hypersensitivity analogous to the human-to-human transfer, known as the Prausnitz-Küstner reaction.¹⁹ Heating the worker's serum at 56° C for 4 hours resulted in a negative test result.

Antibody titers for IgE and IgG were similar for all 4 years. The results of ELISA for IgE and IgG against F-HSA are shown in Fig. 1, *a* and *b*. The IgE antibody titer was 1:10, and the IgG antibody titer was 1:100. The antibody binding was specifically inhibitable by F-HSA. (not shown).

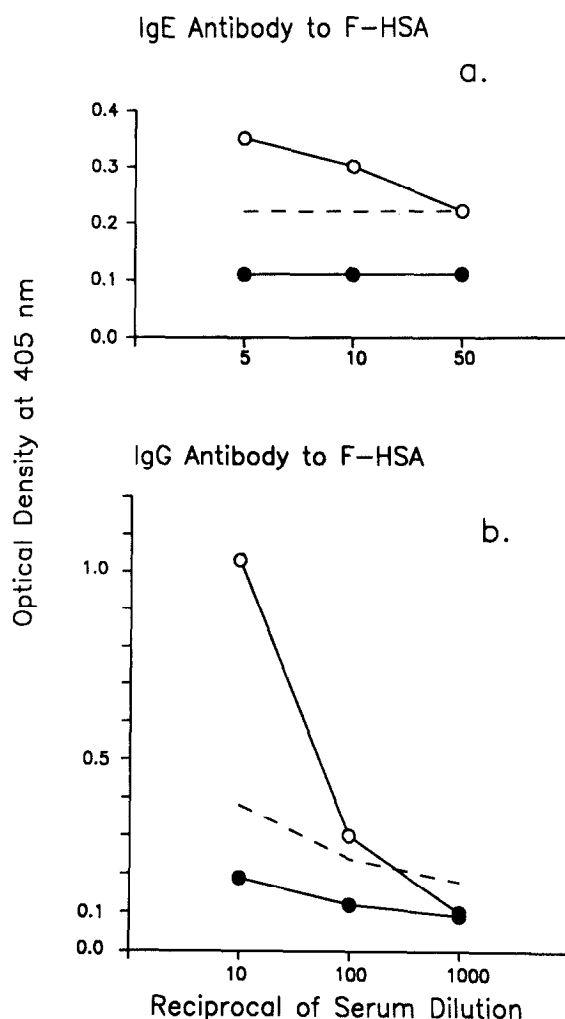


FIG. 1. *a*, ELISA for IgE against F-HSA. The worker (○—○) has a titer of 1:10. The mean of normal controls (●—●) and twice that value (---) are also shown. *b*, ELISA for IgG against F-HSA. The worker (○—○) has a titer of 1:100. The mean (●—●) and twice the mean (---) of normal controls are also shown.

Inhalation challenges

The workers' baseline pulmonary function test results, including results of flow-volume loop, were normal. His methacholine challenge was negative with 25 mg/ml, which was the maximum concentration tested. Formaldehyde challenges on successive days with <0.3, 1, 3, and 5 ppm were negative. Values for the FEV₁, before and after challenge, are shown in Table I.

Overall assessment

After review of the clinical, immunologic, inhalation challenge, and industrial hygiene data, the following conclusions were reached. The worker had a history compatible with asthma induced by

TABLE I. Formaldehyde challenge results: Maximum FEV₁ before and after exposure

	Formaldehyde concentration			
	<0.3	1.0	3.0	5.0
Before exposure	3.93	3.68	3.92	3.89
15 min	3.87	3.98	3.78	3.85
30 min	3.89	3.92	3.78	3.82
1 hr	3.76	3.88	4.07	4.08
3 hr	4.03	3.88	3.87	3.81
6 hr	3.63	3.84	3.97	3.85
24 hr	3.68	3.92	3.89	ND

Concentrations are expressed in parts per million $\pm$ 20%.

ND, Not done.

formaldehyde exposure such as when a pipe leaked, which occurred infrequently in the plant. The worker had clear immunologic evidence of IgE against F-HSA but had a negative inhalation challenge at 5 ppm for 20 minutes. It is possible that a higher exposure level might result in a positive challenge. Therefore our recommendation was for the patient to avoid high levels of formaldehyde exposure (>5 ppm) such as might occur when a pipe leaks.

DISCUSSION

The reason for the negative inhalation challenge in this worker with a compatible history and immunologic evidence of IgE to F-HSA is not clear. It is well known that only a portion of individuals with a positive skin test result to an aeroallergen such as cat dander actually have symptoms when exposed to that aeroallergen; therefore, a positive immunologic test result to a given aeroallergen is insufficient to make a diagnosis of asthma induced by that aeroallergen.²⁰ Compatible clinical information must also be present. In this worker with a compatible history, the result of the challenge could be negative if the worker's symptoms were not immunologically mediated bronchospasm but were in fact an irritant syndrome. Another possibility is that the concentrations that we used in the challenges were not sufficient to reproduce IgE-triggered asthma because the formaldehyde was inactivated before it reached the lower airway. We followed the protocol of Frigas et al.⁶ and exceeded their protocol with an additional, higher concentration (with approval of the Institutional Review Board). The possibility that some higher concentration of formaldehyde might induce asthma in this patient is not excluded.

The worker's negative methacholine challenge deserves comment. Although most subjects with

occupational asthma do have positive methacholine challenge test results, it has been reported that occupational asthma can occur even with a negative methacholine challenge if the worker has been removed from exposure.^{21, 22} In short, the negative methacholine challenge does not preclude asthma, occupational or otherwise.

There are reports of workers with positive formaldehyde challenges in the literature.³⁻⁵ Because no immunologic evaluation was reported, it is unclear whether these were mediated by an immunologic, irritant, or other mechanism. Monkeys challenged with 2.5 ppm of formaldehyde have been reported to have airway obstruction; this obstruction was worse in animals that were more hyperreactive to methacholine.²³ This suggests that formaldehyde might act as an irritant in animals with underlying airway hyperreactivity; this would be analogous to SO₂ challenge in human beings with asthma.²⁴

There has been one report of symptoms consistent with anaphylaxis after intravenous exposure to formaldehyde used to sterilize dialyzers.²⁵ We are unaware of clearly documented reports of IgE-mediated disease that is transmitted via the respiratory route of sensitization. The significance of this report is that it includes an immunologic evaluation, whereas other reports of formaldehyde-induced asthma include only challenge data. After a decade of studying individuals exposed to formaldehyde, their clinical syndromes, and immunologic results, we conclude that immunologically mediated asthma caused by formaldehyde is extremely rare, if it exists at all.

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