Serum IgE and IgG to formaldehyde-human serum albumin: Lack of relation to gaseous formaldehyde exposure and symptoms

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Fifty-five subjects were studied to determine if the presence of IgE or IgG antibodies to formaldehyde (F)-human serum albumin (HSA) was associated with exposure to gaseous F or with respiratory or conjunctival symptoms from such exposure. The study population included cohorts exposed to F in the workplace, smokers, and normal subjects. IgE antibody specific for F-HSA was detected by ELISA in three subjects; immediate-type skin testing was negative in two of these subjects, and not interpretable because of dermatographism in one subject. One of these subjects had a history of respiratory symptoms when the subject was working in a histology laboratory that contained ambient F and xylene; a respiratory challenge with F at concentrations of up to 2 ppm failed to produce respiratory symptoms or significant changes in pulmonary function. Serum from the three subjects with IgE to F-HSA by ELISA failed to passively transfer skin reactivity to F-HSA to rhesus monkey recipients. These three subjects and two other subjects had IgG to F-HSA by ELISA, although this was of generally low titer. We could not define a relationship between the presence of antibodies and (1) a history of F exposure or (2) a history of adverse respiratory or conjunctival symptoms from F. This study is a continuum of 5 years of study in our laboratory attempting to define allergy to gaseous F, and the current study does not support an immunologic basis for respiratory or conjunctival symptoms from gaseous F exposure. Based on the findings of this and our other studies, it is possible that clinical IgE-mediated allergy to gaseous F does not exist, or if it does exist, it is extremely rare. (J ALLERGY CLIN IMMUNOL 1991;87:48-57.)

Exposure to F may result from a variety of occupational and nonoccupational settings. There has been concern about the potential adverse health effects of F, and this concern dates back several decades.[1-14] Health concerns about F range from its potential carcinogenicity suggested by the development of nasal carcinoma in rats and mice,[8] its well recognized effects causing irritant and allergic dermatitis, irritation of the conjunctiva and respiratory tract,[7, 9-12] and the possibility of immunologic sensitization.[13, 14]

Abbreviations used

F:	Formaldehyde
HSA:	Human serum albumin
PBS:	Phosphate-buffered saline
OD:	Optical density
TMA:	Trimellitic anhydride

Although antibodies to F have been described[15-25] and respiratory responses including asthma to F exposure suggestive of sensitization have been reported,[7, 26, 27] it has not been established that antibodies to F can mediate immunologic disease from gaseous F exposure. To address this issue, we studied subjects with a history of exposure to gaseous F in laboratory environments, cigarette smokers (who have exposure to gaseous F in cigarette smoke[27-30]), and nonsmokers without known occupational exposure to gaseous F. We examined (1) whether exposure to gaseous F was

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associated with the development of antibodies to F-HSA and (2) whether the presence of antibodies correlated with a history of respiratory and conjunctival symptoms from gaseous F.

METHODS

Subjects

Fifty-five volunteer subjects were studied. Participation was in accordance with informed consent provisions approved by the Institutional Review Board of Northwestern University. Cohorts of hospital histology technicians, internal medicine residents, and pathology residents were recruited at Northwestern University, Chicago, by oral presentations to these target groups by one investigator (M. D.). Cohorts of current smokers, normal control subjects without a history of defined exposure to formaldehyde, and other subjects with known workplace formaldehyde exposure were recruited by posted notices at Northwestern Memorial Hospital and Northwestern University Medical School. Subjects were excluded from study if they had serious medical illnesses; only one subject was so excluded. Each subject was evaluated for the presence of upper or lower respiratory tract and ocular symptoms by completing a questionnaire with the assistance of one physician (M. D.) who, when it was necessary to clarify history, pursued responses by asking an additional series of questions and recording subject responses.

Independent assessments of possible F-related illness based on questionnaire responses were made by two physician investigators (M. D. and R. P.) who then jointly established a presumptive diagnosis about the nature of possible F-related illness before serologic results were unblinded. The assessment of respiratory or ocular symptoms was based on previously described methodology used in analyzing allergic versus irritant reactions to the industrial antigenic and irritant chemical TMA.[31, 32] Briefly, historical elements judged to suggest an irritant history included onset of symptoms on first exposure to elevated F levels, symptoms only with higher levels of F at which many individuals also experience irritant symptoms, and immediate onset of symptoms with each exposure. Historical elements judged to suggest a possible allergic history included onset of symptoms only after repeated F exposure (latent period of sensitization), symptoms at lower levels of F at which most individuals do not experience symptoms, and delayed onset of symptoms with each exposure.

Serum samples

Blood was drawn from each subject for serology to F-HSA and HSA alone and, in some cases, passive cutaneous sensitization of rhesus monkeys. Serum was separated from cells and stored at −20° C until antibody assays were performed.

Preparation of F-HSA conjugate

This method has been previously described.[23] Briefly, F-HSA was prepared by mixing a quantity of a stock F solution containing 380 mg/ml of F (Fisher Scientific, Fair Lawn, N.J.) with HSA (approved by Food and Drug Administration) (Albuminar-25, Armour Pharmaceutical Co., Kankakee, Ill.) in PBS, pH 7.4. One milligram of HSA was exposed to 1 mg of F. The mixture was incubated for 30 minutes at 37° C and was then dialyzed extensively against PBS. The F-HSA was then sterilized with a 0.2 μm filter (Millipore Corp., Bedford, Mass.). Additionally, HSA without F added was brought through all steps (referred to as sham-HSA) and served as control HSA for serologic assays and skin testing.

To determine whether conjugation occurred, Immunotec II, immunoelectrophoresis agarose plates (Calbiochem Corp., San Diego, Calif.), were used. Immunoelectrophoresis was performed by electrophoresis of HSA or F-HSA and developed with precipitating rabbit anti-HSA (Calbiochem Corp.). This analysis demonstrated altered electrophoretic migration of F-HSA as compared with HSA and was evidence that F was conjugated to HSA. Additionally, the number of free amino groups present in the conjugate and sham-HSA was determined according to the method of Snyder and Sobocinski.[33]

ELISA method for detection of IgE and IgG to F-HSA and HSA

The technique used was adapted from ELISA protocols previously described.[34, 35] The wells of polystyrene Immulon micro-ELISA plates (Greiner & Sons, Nürtingen, West Germany) were coated with F-HSA (or sham-treated HSA) at a concentration of 100 μg/ml in carbonate buffer (pH 9.6). A 200 μl volume was used for this and all subsequent steps. The plates were incubated overnight at 4° C, emptied, and then washed three times with PBS containing 0.05% Tween 20 (Sigma Chemical Co., St. Louis, Mo.). The washing procedure was repeated between all steps in the assay. Appropriate dilutions of the worker or control sera in PBS-Tween were added to the plates and then incubated for 60 minutes at 37° C. Rabbit antihuman IgG (Calbiochem Corp.), diluted 1:1000 in PBS-Tween, or rabbit antihuman IgE (Accurate Chemical and Scientific Corp., Westbury, N.Y.), diluted 1:500, was added and incubated for 45 minutes at 37° C. Then, alkaline phosphatase–conjugated goat antirabbit IgG (Sigma Chemical Co.), diluted 1:1000 in PBS-Tween, was added and incubated at 37° C for 60 minutes. Finally, p-nitrophenyl phosphate (104 phosphatase, Sigma Chemical Co.), 1 mg/ml in diethanolamine buffer (pH 9.8), was added to each well. The OD of each well was read on a BioTek model EL 312 automated ELISA reader (Bio-Tek Instruments, Inc., Winooski, Vt.) at 405 nm. The reaction was allowed to proceed until the OD of a positive control serum reached a predetermined value. The positive control serum (1) could be inhibited in the ELISA by F-HSA, (2) could passively transfer immediate cutaneous reactivity to rhesus monkeys (performed before these studies), (3) lost the ability to be passively transferred by heating the serum for 1 hour at 56° C, and (4) was obtained from a subject with respiratory symptoms from F exposure but who did not agree to further study. The end point dilution was defined as the last dilution of worker serum having an

TABLE I. Characteristics of cohort groups

Cohort	No.	Age		Sex
		Mean	± SEM	(% F)
Histology technicians	10	32.9	1.81	100
Medical residents	10	27.4	0.07	30
Pathology residents	9	30.1	0.75	44
Smokers	11	35.7	0.28	64
Misc F exposure	5	44.8	7.20	0
Normal subjects	10	33.3	3.71	70
All subjects	55	33.2	1.26	56

E, eye; *L*, lung/lower respiratory; *N*, nose; *S*, skin; *misc*, miscellaneous.
*No recollection of F exposure by subjects.

OD greater than twice the mean of the normal control sera.

ELISAs were performed by individuals who had no knowledge of clinical information. Assays of serum samples were determined on at least two occasions and found to be reproducible.

ELISA-inhibition studies

Inhibition studies were performed on the positive control serum and on the sera of subjects who had binding (greater than twice that of normal control sera) to F-HSA in the IgE or IgG assay. PBS or 1 mg of inhibitor (F-HSA, HSA, or as a nonspecific antigen, ragweed antigen E) was added per milliliter of serum and was incubated for 30 minutes at 37° C. The ELISA was performed exactly as described above except that the serum samples preincubated with inhibitor were added to the microtiter wells instead of serum alone.

Cutaneous testing

Three subjects who had detectable levels of IgE to F-HSA (IgE-F-HSA) by ELISA had immediate-type skin testing performed with F-HSA, sham-HSA, histamine phosphate as a positive control, and normal saline as a negative control. Concentrations used for prick and intradermal testing, respectively, were F-HSA (5 mg/ml and 1, 10, and 100 μg/ml), HSA (5 mg/ml and 1, 10, and 100 μg/ml), and histamine phosphate (5 and 0.275 mg/ml).

Passive cutaneous transfer

Passive cutaneous transfer was performed in rhesus monkeys anesthetized with pentobarbital using the Prausnitz-Küstner technique.[36] In brief, 0.1 ml of twofold increasing dilutions of serum from patients with specific IgE to F-HSA or nonatopic human serum (as a control) were injected intracutaneously. Forty-eight hours later, 4 ml of 0.5% Evans blue dye was administered intravenously. F-HSA and HSA were injected into unsensitized sites and demonstrated that the monkey did not have reactivity to either reagent alone. The skin sites previously sensitized with serum were challenged with F-HSA. A positive reaction was a reaction displaying a deep blue color at least 10 mm in diameter occurring within 30 minutes. A negative reaction had no bluing.

Measurement of gaseous F levels

F and xylene levels (where these were present) in the work environment of histology technicians and pathology residents working at Northwestern Memorial Hospital, Chicago, were determined by an industrial hygiene company with a Wilks Moran 1A portable analyzer that has a sensitivity in the range of 0.01 ppm for testing F and 0.6 ppm for testing xylene.

Respiratory challenge

During a 2-day period, single-blinded, respiratory challenges with gaseous F were performed with a system adapted from Frigas et al.[37] F gas at different concentrations or ambient air (acting as a placebo) was delivered to the subject during 20 minutes through a face mask that covered the mouth and nose. Spirometry, including flow-volume loops, were measured in triplicate before and at 0, 15, and 60 minutes after challenge. On day 1, placebo, 0.1 and 1 ppm challenges were performed. On day 2, 1 and 2 ppm challenges were performed. Calculated F concentrations based on airflow across the gas source, diluent flow, and gas source temperatures were found to be within 10% of the value measured in the inspired gas stream by use of Air Scan STEL F monitors (Crystal Diagnostics, Inc., Woburn, Mass.).

Statistical analyses

A one-way analysis of variance was performed with SPSS/PC+ software (SPSS, Inc., Chicago, Ill.).

RESULTS

Characteristics of cohort groups, including history of F exposure and smoking history, are tabulated in Table I. Specific aspects of these data are summarized below.

Percent with symptoms				Years of occupational F exposure		Tobacco-pack years	
E	L	N	S	Mean	±SEM	Mean	±SEM
80	80	90	40	12.45	2.46	0.50	0.50
30	0	10	0	0.38	.04	0	
89	11	55	0	3.21	0.44	0.67	0.55
9	9	9	0	0		16.41	2.99
40	40	40	0	18.34	6.16	16.4	6.74
*	*	*	*	0		0	
				4.53	1.09	4.97	1.28

Subject characteristics

There were 31 female and 24 male subjects in the study population with a mean age of 33.2 ± 1.3 (SEM). In comparing cohorts (Table I), histology technicians had the greatest duration of workplace F exposure and the highest percentage of subjects reporting symptoms from exposure.

History and clinical assessment

Of 34 subjects who reported occupational exposure to F, 25 reported adverse symptoms; eye (conjunctivitis) symptoms were reported by 21, nose (rhinitis) symptoms by 17, lung or lower respiratory symptoms (e.g., cough, wheezing, or shortness of breath) by 11, and four histology technicians reported skin rashes (Table II). Among these 25 subjects reporting adverse symptoms from F, 17 had clearly irritant symptoms by history, and the remaining eight subjects had symptoms consistent with an irritant basis, although some elements of the history suggested a possible allergic basis.

F-HSA conjugate

The F-HSA conjugate was assayed and found to have 23 free amino groups (as compared to 58 unbound free amino groups on HSA), demonstrating haptenization with F.

ELISA and ELISA-inhibition studies

No subjects had ELISA results, suggesting IgE or IgG antibody against HSA (IgE-HSA or IgG-HSA). Three subjects (Nos. 9, 13, and 36) had IgE-F-HSA. These three subjects and two other subjects (Nos. 20 and 28) had detectable IgG to F-HSA (IgG-F-HSA) without IgG-HSA.

Inhibition studies were performed with serum from all subjects who had antibody activity against F-HSA by ELISA. ELISA for IgE and IgG were inhibited completely by serum preincubation with F-HSA in all subjects and were partially inhibited by HSA in only one subject. Ragweed antigen E failed to inhibit the ELISA activity of any subject. This finding demonstrated that the antibodies detected against F-HSA were specific for F-HSA.

Comparison of IgE-F-HSA, IgG-F-HSA with gaseous F exposure, and history of symptoms

The presence of specific IgE-F-HSA or IgG-F-HSA did not relate to presumptive F exposure based on duration of gaseous F exposure or with pack years of tobacco smoking (Table II). The presence of either specific IgE-F-HSA or IgG-F-HSA did not correspond to the presence of respiratory, conjunctival, or skin symptoms from F judged to be either irritant or possibly allergic (Table II).

Skin testing

The three subjects who had IgE-F-HSA (Nos. 9, 13, and 36) had immediate-type skin testing to F performed; two had negative skin tests to F-HSA and HSA (with positive histamine controls) and one (No. 9) was dermatographic, and skin test results could not be interpreted.

Passive cutaneous transfer

Serum from subjects with specific IgE-F-HSA (Nos. 9, 13, and 36) all failed to confer passive cutaneous reactivity to F-HSA or HSA in rhesus monkey skin.

Measured workplace levels of F

Representative measurements of exposure (15 minutes short-term exposure limit [STEL]) to gaseous F were 0.2 to 0.64 ppm for pathology residents, 0.64 ppm (two determinations) for histology technicians, and 0.6 to 11 ppm for an autopsy diener (subject No.

TABLE II. Symptom history, antibody titers, skin testing, and final assessment

Subjects	Symptoms with F	Physician assessed history		
		P1	P2	C
Histology technicians				
1	ENL	I	I	I
2	EN	I	I	I
3	NL	I	I	I
4	NLS	I, PA	I, PA	I, PA
5	ELS	I	I	I
6	ENLS	I, PA	I, PA	I, PA
7	ENLS	I, PA	I, PA	I, PA
8	ENL	I, PA	I, PA	I, PA
9†	ENL	I, PA	I, PA	I, PA
10	EN	I	I	I
Medical residents				
11	EN	I	I	I
12		N	N	N
13		N	N	N
14		N	N	N
15		N	N	N
16		N	N	N
17		N	N	N
18	E	I	I	I
19	E	I	I	I
20		N	N	N
Pathology residents				
21	EN	I	I	I
22	EN	I	I	I
23	N	I	I	I
24	EN	I	I	I
25	ENL	I, PA	I, PA	I, PA
26	E	I, PA	I	I
27	E	I, PA	I	I
28	E	I	I	I
29	E	I	I, PA	I, PA

E, eye; *N*, nose; *L*, lung; *S*, skin; *P1*, physician 1; *P2*, physician 2; *C*, conference opinion; *I*, irritant; *PA*, possible allergic; *N*, no symptoms.
*When a second antibody titer is listed, this was of sera drawn 1 year after original study sample.
†Subject No. 9 had negative respiratory challenges with F up to 2 ppm.

53). Representative measurements of exposure to xylene ranged from <3 to 58.5 ppm for these subjects.

Aerosol F respiratory challenge

Subject No. 9 who had both IgE to F-HSA and a history of eye and respiratory symptoms in association with workplace exposure to F (itchy/irritated eyes, nasal stuffiness, runny nose, cough, and shortness of breath) was challenged and had no symptoms or significant change in pulmonary function tests after any of the four F challenges.

Statistical analyses

No significant difference in incidence of IgG or IgE antibody against F-HSA was found between the groups with analysis of variance (IgG: $F_{(5,49)} = 0.4604$; IgE: $F_{(5,49)} = 0.8190$).

DISCUSSION

Although antibodies to F-HSA were found in some subjects of our study population, we could not define a relation between the presence of IgE or IgG to F-HSA and a history of F exposure or a history of adverse respiratory or conjunctival symptoms from gaseous F exposure. Based on the well-defined model of clinical and immunologic syndromes resulting from exposure to TMA,[31, 32] reactive chemicals of small molecular weight (<1000 daltons) are believed to cause allergic respiratory disease by combining with self-proteins to form hapten-self-protein conjugates that

Antibody titers*		Immediate-type F skin testing	Other history
IgE-F-HSA	IgG-F-HSA		
0	0		
0	0		
0	0		Asthma, 26 yr
0	0		
0	0		History of COPD
0	0		Previous F exposure >15 ppm
0	0		Bronchitis
0	0		
5, 5	100, 100	Dermatographism	Seasonal rhinitis
0	0		
0	0		
0	0		Seasonal rhinitis
10, 5	>1000, 100	Negative	
0	0		
0	0		Asthma, 10 years
0	0		Volunteer fireman
0	0		
0	0		Seasonal rhinitis
0	0		
0	100		Seasonal rhinitis
0	0		Prior rhinitis
0	0		
0	0		Seasonal rhinitis
0	0		
0	0		
0	0		
0	0		Stopped smoking, 10 yr
0	10		
0	0		Stopped smoking, 5 yr

are immunogenic. In immunologically mediated syndromes induced by TMA, such as asthma and rhinoconjunctivitis, antibodies to trimellitic A hapten-self-protein conjugates reflect TMA exposure and correlate with disease.[31, 32] Because F is also a reactive chemical of low molecular weight (30 daltons), it would be presumed that if gaseous F were capable of causing immunologic respiratory or other syndromes, the presence of antibodies to F-HSA would relate to gaseous F exposure or with a history of adverse symptoms from F exposure in a manner analogous to TMA. Because we did not find this relationship, we must consider that the symptoms from F reported by our subjects were not immunologically mediated but were caused by irritant effects from F or were caused by other chemicals coexisting with F.

In contrast to our cohorts of histology technicians, pathology residents and miscellaneous F-exposed subjects (all subjects who were being regularly exposed to gaseous F at the time their sera were drawn for antibody studies), the cohort of internal medicine residents had received their significant F exposure 3 to 5 years before this study (during anatomy and histology classes in medical school). Nevertheless, their antibody levels were similar to levels in the other cohorts. This may indicate that antibody levels to F-HSA persist once they develop.

Because we found no relation between IgE to F-HSA and either F exposure or symptoms from F exposure, the IgE-F-HSA detected in our subjects does not appear to have immunopathologic significance. In addition, both subjects in whom interpretable immediate-type skin testing was performed had negative skin tests despite the presence of IgE specific

TABLE II. Cont'd

Subjects	Symptoms with F	Physician assessed history		
		P1	P2	C
Smokers				
30		N	N	N
31		N	N	N
32		N	N	N
33		N	N	N
34		N	N	N
35	ENL	I	I	I
36		N	N	N
37		N	N	N
38		N	N	N
39		N	N	N
40		N	N	N
Normal subjects				
41		N	N	N
42		N	N	N
43		N	N	N
44		N	N	N
45		N	N	N
46		N	N	N
47		N	N	N
48		N	N	N
49		N	N	N
50		N	N	N
Misc F exposures				
51	ENL	I, PA	I, PA	I, PA
52		N	N	N
53		N	N	N
54	NL	I	I	I
55	E	I	I	I

for F-HSA by ELISA. Since immediate type skin tests are generally considered as sensitive or more sensitive than in vitro tests for the detection of IgE antibody,[38] the absence of positive skin tests in these individuals calls into question whether the IgE-F-HSA detected in vitro has pathologic significance. Furthermore, the failure of sera from the three subjects with IgE-F-HSA to sensitize passively monkey skin to F-HSA also fails to support a biologic role for IgE-F-HSA.[39] These results also raise the issue of whether or not commercially available assays for antibodies to F have any value in making assessments about clinical disease; the detection of antibodies alone does not indicate disease.

Our data do suggest that IgG antibodies to F-HSA tend to occur in association with IgE antibodies to F-HSA. Because the titers of IgG-F-HSA generally observed (1:100) are lower than IgG titers associated with immunologic disease to other antigens[40] and because IgG-F-HSA and symptoms were not related, there is no indication that the IgG antibody detected is of pathologic significance.

It is possible that some symptoms ascribed to F by our study subjects were related to concomitant exposure to other agents that act as irritants. For example, the histology technicians in the study were also exposed at times to xylene, alcohol, and toluene. Experiments in which F has been mixed with other irritants have yielded lower irritation thresholds than experiments for formaldehyde alone.[41] This may explain why the histology technician in our study who had respiratory challenges with F at levels (1 to 2

Antibody titers*		Immediate-type F skin testing	Other history
IgE-F-HSA	IgG-F-HSA		
0	0		Stopped smoking, 17 yr
0	0		Seasonal rhinitis
0	0		
0	0		
0	0		Daily coughing
0	0		
5, 5	100, 100	Negative	Seasonal rhinitis
0	0		Rhinitis/baker
0	0		Rhinitis, bronchitis
0	0		Rhinitis
0	0		Diabetes
0	0		
0	0		Seasonal rhinitis
0	0		
0	0		Occasional cigarette, >25 yr
0	0		Asthma, rhinitis
0	0		
0	0		Asthma in past
0	0		
0	0		Rhinitis
0	0		
0	0		Autopsy assistant, stopped smoking
0	0		Anatomy laboratory technician, rhinitis, stopped smoking, 5 yr
0	0		Autopsy diener
0	0		Autopsy diener
0	0		Surgical MD on pathology rotation for 6 wk

ppm) that were above levels measured in her workplace (0.64 ppm) had no symptoms or significant pulmonary function changes during controlled F challenges. Skin rashes reported by four histology technicians in our study also may have been related to exposure to these other agents or to gaseous F or formalin.

Studies of the antigenicity of F-treated proteins date back more than 70 years.[15-24] It is reported that immunologic sensitization of the skin may occur by F acting as a contact allergen and hapten, combining with self-proteins and inducing type IV hypersensitivity.[42-44] Patients undergoing dialysis exposed to F intravenously by use of F-sterilized dialyzers can develop anti-N-like antibodies and antibodies to F and F-HSA.[19-22] Dogs exposed to intravenous F solutions develop antibodies of various immunoglobulin classes to F-albumin conjugates.[23] IgE antibodies to F-HSA have been described in a patient who developed anaphylactic shock during hemodialysis with an F-sterilized dialyzer.[24]

However, studies examining gaseous F exposure have yielded much different results. In subjects with asthma exposed to gaseous F and urea formaldehyde foam insulation off products, anti-F red blood cell antibodies could not be detected.[45] Although there has been a case report of two subjects who had F-specific IgE antibodies by RAST and a history of respiratory symptoms with workplace exposure to F, neither controlled respiratory challenges nor skin testing with F was performed.[46] In 13 patients with asthma suspected to be related to F who did have controlled bronchial

challenges, F levels of 3 ppm or less failed to cause significant decreases in pulmonary function.[37] Two renal dialysis nurses (exposed to gaseous F by exposure to formalin used as a sterilizing agent) have been reported to have positive provocative challenges to formalin spills[7] and IgE antibodies to F-HSA[22]; however, the F levels generated for challenges (3 to 6 ppm) were high and could have caused asthmatic responses on an irritant basis.[7] In a report of 58 subjects exposed to gaseous F, only one subject had F-specific IgE antibodies, and this subject did not have any symptoms from F workplace exposure.[25] In yet another study of 37 workers exposed to gaseous F, no workers had IgE or IgG antibody to F-HSA.[47]

With the results of this study, there is mounting evidence that immunologically mediated rhinitis, conjunctivitis, or asthma from F must be of extremely low frequency, if it exists at all. This study completes 5 years of investigation in our laboratory beginning with application of modern ELISA technology for detection of antibodies in humans receiving F intravenously.[22] Despite evaluation of individuals in industrial and other situations with F exposure, we have not identified any patient with IgE-mediated allergy to gaseous F with criteria from the well-established models of immunologic respiratory disease caused by common aeroallergens or TMA.

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