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Human Antibodies against Formaldehyde-Human Serum Albumin Conjugates or Human Serum Albumin in Individuals Exposed to Formaldehyde

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Abstract. Sera from patients undergoing hemodialysis with formaldehyde (F)-sterilized dialyzers were studied to determine if antibodies against F conjugated to human serum albumin (HSA) could be detected. F-human serum albumin (F-HSA) conjugates were prepared using ratios of F to HSA that did not precipitate the HSA. The F-HSA conjugates migrate differently electrophoretically than HSA with an increased negative charge of F-HSA as compared with HSA. The F-HSA was used in an ELISA. The results demonstrated that in certain sera, IgG, IgM, IgA and IgE antibodies against F-HSA could be measured. In the highest titered sera, it was shown that the IgG antibody was not directed against F alone or F-lysine but against an antigenic grouping of F-HSA. No correlation of either IgG or IgE antibodies with immune complex or allergic reactions was found in this series of dialysis patients. Some sera from dialysis patients had antibody activity against HSA. Sera from 2 physicians with rhinitis after F exposure had no antibody activity against F-HSA or HSA. Two nurses with a history of F-induced asthma had no IgG antibodies but did have IgE antibodies against F-HSA and HSA. This spectrum of immunologic responses is analogous to responses in dogs immunized with F or F dog albumin. We have not been able to identify anti HSA antibodies in patients reactive to other hapten-HSA compounds and it is suggested that anti HSA antibodies in F-exposed humans may relate to the F exposure.

Introduction

Formaldehyde (F) is a chemical with high volume production and multiple uses. The result is human exposure in occupational and other environmental areas. The questions of effects of F exposure on human health have been topics of past and current research. The health concerns range from the occurrence of nasal cancer in rats which has created concern about carcionogenicity, toxicity, and primary irritant symptoms to allergic sensitization [1]. Use of the latter term is best restricted to human disease states where there is evidence of immunologic reactivity against the antigen demonstrated by in vitro or in vivo immunoassays or strong presumptive evidence of immunologic mechanisms as identified in certain human allergic drug reactions [2]. Human allergy to F manifested by contact dermatitis has been established [1]. This type of hypersensitivity is presumably the result of lymphocyte-mediated reactivity [2] although in vitro demonstration of responses by lymphocytes have not yet been shown. Asthma [3] and rhinitis [4] due to F exposure have been reported but no demonstration of circulating antibodies against F consistent with immunologic airway disease have been identified. IgE antibodies and possibly IgG antibodies would be expected to be correlated with such airway disease [2]. We have developed dog models of immune response and immunoassays for canine antibodies against F-dog serum proteins. These methodologies were based on previous studies of immunologic responses in humans against inhaled trimellitic anhydride [5].

Antibodies against erythrocytes in patients hemodialyzed with F-sterilized dialyzers have been reported in several studies [6, 7]. There appeared to be a relation of the F exposure to the antierythrocyte response [7].

Table I. Sera analyzed for antibody activity against F-HSA

Serum number	Source of sera	Comments
1–18	patients hemodialyzed with F-sterilized equipment	no definite symptoms correlated with hemodialysis procedures
19	physician, occupational	Describes acute rhinitis after F exposure
20	physician, pathologist	Respiratory symptoms with F. See ref. [4]
21	nurse[1]	Dialysis nurse exposed to F from 1973 to 1976 and from 1976 to 1984 under improved environmental conditions. Asthma related to F exposure. Positive bronchial challenge with F in 1973, 1975 and 1981. Serum obtained in 1984.
22	nurse[1]	Dialysis nurse exposed to F from 1973 to 1976 with asthma. No further F exposure after 1976. Positive bronchial challenge with F in 1973 and 1975 but negative in 1981. Serum collected in 1984.
23–28	laboratory workers control sera	Normal laboratory workers without respiratory symptoms or F exposure

[1] See ref. [3, 17, 20].

Materials and Methods

Serum Samples

These were obtained from patients undergoing hemodialysis with F-sterilized dialysis equipment, 2 physicians with F-rhinitis, 2 nurses with F-asthma and a normal control population of research laboratory personnel. These sera are listed in table I.

Preparation of F-Human Serum Albumin (F-HSA)

F-HSA was prepared by methods described to prepare F-dog serum albumin (DSA) [8]. F-HSA was prepared by adding varying dilutions of a stock F solution containing 380 mg/ml F (Fisher Scientific, Fair Lawn, N.J.) to chromatographically purified HSA (Cappel Laboratories, Cochranville, Pa.) in phosphate-buffered saline (PBS), pH 7.4. One milligram of HSA was exposed to 10, 1 or 0.1 mg of F. The mixtures were incubated for 30 min at 37 °C and then dialyzed extensively in PBS. The F-HSA was then sterilized with a 0.2-μm filter (Millipore Corp., Bedford, Mass.). Spectrophotometric analysis yielded a spectrum identical to HSA alone.

Immunoelectrophoresis

To determine whether conjugation had occurred Immuno-tech II IEP agarose plates (Calbiochem-Behring, La Jolla, Calif.) were used. Immunoelectrophoresis was performed by electrophoresis of HSA or F-HSA and developed with precipitating rabbit anti HSA (Calbiochem-Behring, La Jolla, Calif.). This analysis showed both the altered electrophoretic migration of F-HSA as compared with HSA which is evidence that F was conjugated to HSA.

Enzyme-Linked Immunosorbent Assay (ELISA)

The ELISA procedure was performed according to a modification of methods previously described [9, 10]. All steps were carried out at 37 °C to prevent nonspecific reactions due to cryoglobulins which became apparent when the canine antibody studies were done [8]. Immulon flat-bottomed micro ELISA plates from C.A. Greiner and Son, (Nürtingen, FRG) were coated with a 1:100 dilution of F-HSA in carbonate buffer (pH 9.6), 200 μl per well and incubated for 30 min. The plates were then washed with PBS-Tween (Sigma Chemical Co., St. Louis, Mo.) (0.05%). Dilutions of test sera were made in PBS-Tween at 37 °C and incubated for 30 min before adding to the microplate. Two hundred microliters of the serum dilutions were added to individual wells and the plates were incubated for 30 min. Plates were washed 3 times in PBS-Tween and a previously determined dilution of rabbit anti human IgG or IgE was added to each well, and plates were incubated for 30 min. After washing again, 200 μl of alkaline phosphatase-conjugated goat anti rabbit gamma globulin (Sigma) was added and plates were incubated for 1 h. A final washing was done and *p*-nitrophenyl phosphate (Sigma 104 Phosphate, 1 mg/ml) in 10% diethanolamine buffer, pH 9.8, was added and incubation was carried out at room temperature for 30 min. The IgE antibody assay was incubated an additional 30 min at 37 °C. The reaction was stopped with 50 μl of 3 *M* NaOH and the optical density of each well was then read at 405 nm on an Artek Automatic ELISA Reader (Farmingdale, N.Y.) ELISA titer endpoints were read as the last dilution at which the test antiserum had an optical density reading which was still 2 times that of the control serum.

Inhibition Studies

Inhibition studies were carried out on serum 1 which had a high titer of anti F antibodies. One milligram of inhibitor (F, F-HSA, F-DSA, F-lysine, HSA or PBS as control) was added per ml of serum and incubated for 30 min at 37 °C. These samples were then diluted and processed as described for the ELISA.

Results

Characteristics of F-HSA

One milligram of chromatographically pure HSA was exposed to the following quantities of F: 0.1, 1, 10, 100 and 380 mg. The highest concentration of F resulted in precipitation of HSA. Lower concentrations of F resulted in soluble preparations. These were con-

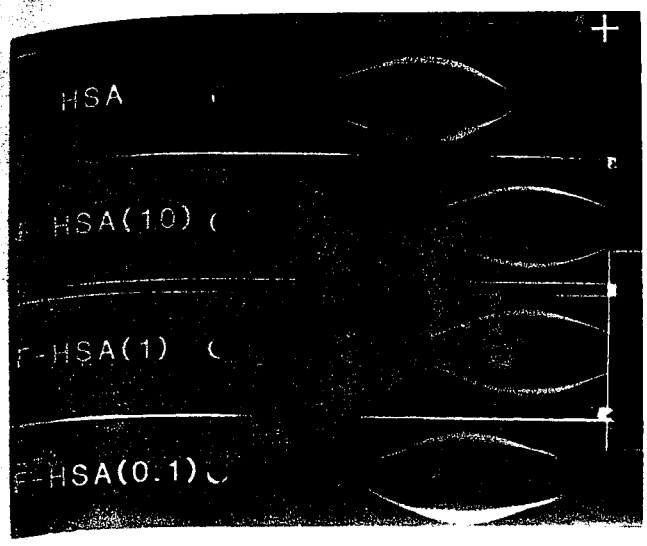

Fig. 1. Immunoloelectrophoresis of HSA and F-HSA when F-HSA was prepared by exposure of HSA to increasing concentrations of F. Precipitin arcs were developed by rabbit anti HSA. The numbers next to F-HSA show the concentration in mg of F to which 1 mg of HSA was exposed.

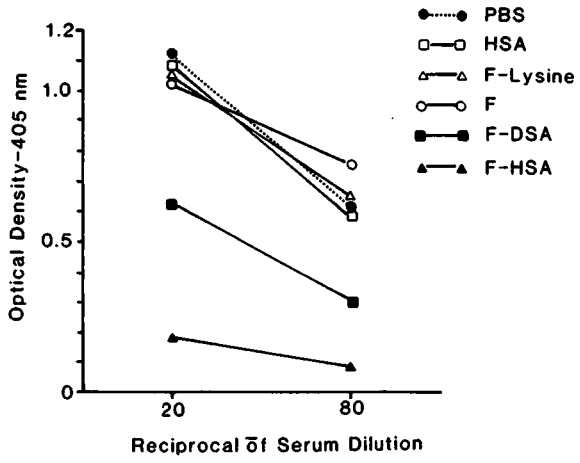

Fig. 3. Specificity of IgG antibody in Serum 1 against F-HSA shown by inhibition analysis with ELISA. Complete inhibition occurs with pre-incubation with F-HSA, partial inhibition with F-DSA and no inhibition with F, F-lysine, or HSA as compared with the control titers.

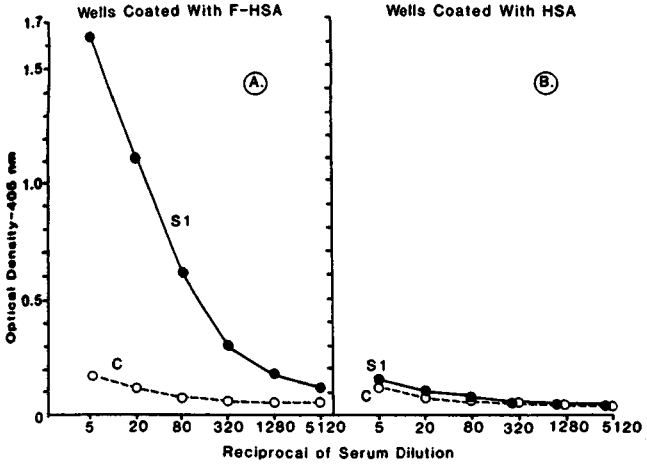

Fig. 2. Analysis of antibody activity in dialysis Serum 1 (S1) against F-HSA compared with serum from normal control (C). **A** IgG antibody against F-HSA, **B** absence of IgG antibody against HSA.

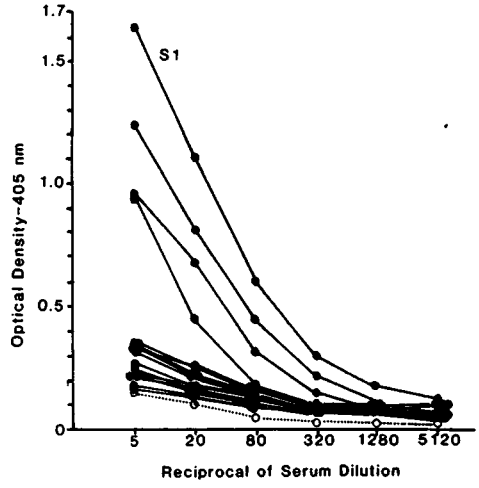

Fig. 4. IgG antibody activity against F-HSA in sera of patients hemodialyzed with F-sterilized dialysis equipment. Serum 1 (S1) is shown in figures 2 and 3. Control (O---O) is the mean of 3 normal sera.

sidered to be F-HSA and the preparations made by exposing 1 mg HSA to 0.1, 1 or 10 mg F were analyzed by immunoelectrophoresis developed with rabbit anti HSA (fig. 1). The results of this study show that the soluble preparations of F-HSA migrated farther toward the anode than HSA. Further, those preparations of F-HSA prepared by exposure to higher concentrations of F migrated more rapidly toward the anode. These are consistent with an increased negative charge of F-HSA preparations which were prepared by exposure of HSA to higher concentrations of F.

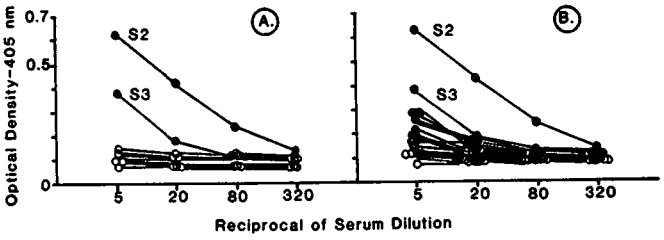

Fig. 5. IgE antibody against F-HSA demonstrated by ELISA. **A** Two hemodialysis patient sera (S2 and S3) (●——●) compared with 5 control sera (O——O). **B** All hemodialysis sera (●——●) compared with control sera (O——O).

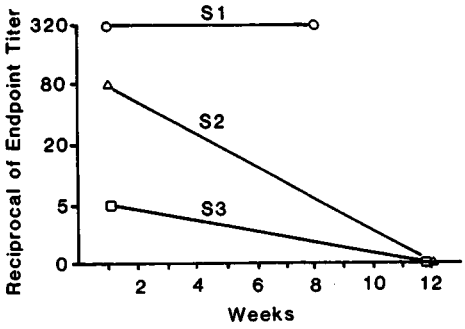

Fig. 6. Persisting IgG antibody titer against F-HSA in serum samples from Serum 1 (S1). Endpoint titer is based on optical density 2 times control level in ELISA. IgE antibody in 2 patients was absent 3 months later (S2 and S3).

IgG Antibody against F-HSA

Preliminary screening of sera from patients hemodialyzed with F-sterilized dialysis equipment and sera from normal laboratory workers indicated that there was antibody activity against F-HSA in sera of some dialysis subjects. The IgG ELISA titration of the patient with the highest antibody level (Serum 1) is shown in figure 2. This serum was studied in detail to demonstrate antibody specificity against F-HSA. As shown in figure 2A, a high antibody level against F-HSA was demonstrated while the control serum showed a totally different curve indicating no antibody activity. Serum 1 showed no antibody activity against the HSA from which the F-HSA had been prepared (fig. 2B).

Specificity of IgG Antibody against F-HSA by Inhibition Analysis

The specificity of IgG anti F-HSA was examined by preincubation of dilutions of Serum 1 with excess amounts of various potentially related antigens to determine if such incubation reduced the antibody titer as determined by ELISA. The results are shown in figure 3. These compare the positive control with F-HSA. The latter resulted in complete inhibition. No inhibition was demonstrated with F alone, F-lysine or the HSA from which the F-HSA was prepard. This indicates that the specificity of IgG antibody in this serum (serum 1) is against a combined antigenic determinant, the configuration of which is contributed to by both F and HSA. Preincubation of antiserum with F-DSA resulted in partial inhibition of ELISA titers against F-HSA.

IgG Antibody in Sera of a Series of Hemodialysis Patients

Serum 1 (table I) had the highest IgG antibody titer against F-HSA. Sera from other patients dialyzed with F-sterilized dialysis equipment were examined and results are shown in figure 4. As is evident, there is a range of antibody titers from no antibody in some hemodialysis patients to the highest level of serum 1. The control is a mean of 3 individual normal sera.

IgE Antibodies against F-HSA

IgE antibody was evaluated in all sera from hemodialysis patients. The results are shown in figure 5. Two sera had IgE antibody against F-HSA and these sera are compared with 5 normal control sera in figure 5A. In figure 5B all sera from hemodialysis patients are compared with 5 normal control sera. Some of the other hemodialysis patients' sera may have low levels of IgE antibody against F-HSA.

Repeat Antibody Titers

IgG antibody and IgE antibodies against F-HSA in sera of dialysis patients with the highest titers were measured several weeks after the initial blood samples were obtained. The results (fig. 6) showed that the IgG antibody level persisted but the IgE antibody had declined.

Antibody Measurements on Sera of Physicians with Severe Rhinitis Subsequent to F Exposure

No IgG or IgE antibody activity against F-HSA was found in sera of the 2 physicians listed in table I.

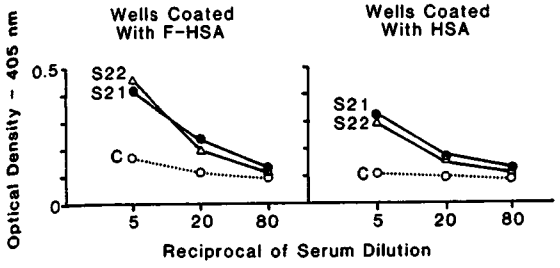

Fig. 7. IgE antibody against F-HSA and HSA in sera (S21 and S22) of nurses with history of F asthma. Control (C) is the mean of 2 normal sera.

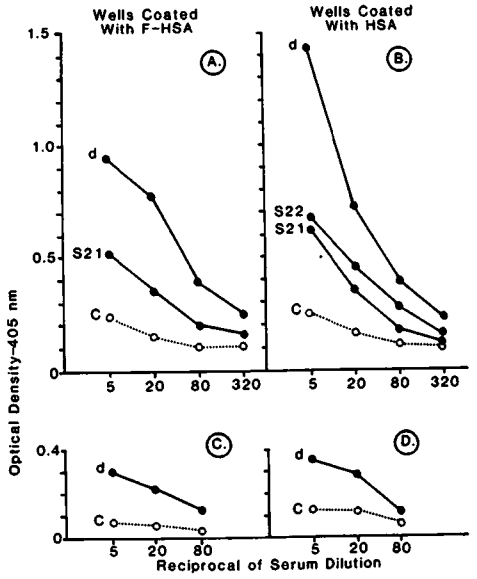

Fig. 8. A and B IgM antibody against F-HSA and HSA, respectively. C and D IgA antibody against F-HSA and HSA, respectively. C is serum from a normal control; D is serum from a representative dialysis patient. For S21 and S22 see table I.

Antibody Measurements on Sera of Nurses with a History of F-Induced Asthma

Neither serum had detectable IgG anti F-HSA. Sera No. 21 and 22 (table I) from the nurses who had a history of F asthma had IgE antibodies against F-HSA and also against HSA (fig. 7).

IgM and IgA Antibody

IgM antibody against F-HSA was found in 1 nurse's and 5 dialysis patients' sera and IgM antibody against HSA in the sera of 2 of the 5 patients and of both nurses (see fig. 8 and table I). IgM antibody against HSA, but not F-HSA, was found in 3 additional dialysis patients. IgA antibody against F-HSA and HSA was found in 9 dialysis sera (fig. 8).

Evaluation of IgG and IgE Antibody against HSA from Other Human Sera against Hapten-HSA Conjugates

For this study we used sera previously studied and reported which had IgE antibody against ethylene oxide-HSA [11] or sera with antibody against trimellitic anhydride-HSA [12]. None of the former (10 sera) had antibody against HSA. One of 30 antisera against TM-HSA had a very low level of antibody against HSA. These results suggest further that the antibody against HSA in F-exposed subjects is related to F exposure.

Discussion

This investigation was initiated to determine if we could identify human antibodies against F-homologous serum proteins following the successful induction and demonstration of such antibodies in dogs [8]. Our initial animal studies had been based on very early studies [13] which had shown antibodies against homologous F serum conjugates in rabbits. Sera from patients exposed to F-sterilized dialysis equipment were selected because in such subjects antibodies related to F have been found against human erythrocytes [6, 7]. The objectives of the study were first to see if antibodies in humans against F-HSA could be demonstrated in hemodialysis patients and secondly to evaluate whether such antibodies might have pathogenetic significance for allergic reactions of patients during hemodialysis as has been suggested for reactions against ethylene oxide-HSA conjugates in some hemodialyzed patients [11]. The third objective was to attempt to establish immunoassays for measuring antibodies against F-human serum proteins for use in human subjects suspected of having allergic reactions to inhaled F in occupational or other exposure and to apply these techniques to human subjects with F-related respiratory symptoms.

The first and third objectives have been achieved. The question whether or not the antibodies against F-

HSA have any relation to symptoms or complications in patients using F-sterilized dialysis equipment remains unanswered. We were not able to make any correlation with these antibodies and clinical abnormalities in this series of hemodialysis patients. The IgG antibodies against F-HSA are present in very low titers as compared with IgG antibodies in such disease states as allergic bronchopulmonary aspergillosis [14] or immune complex disease due to streptokinase [15] using the ELISA assay. In the latter 2 disease states IgG antibodies are considered to be pathogenetic. It has been reported that patients may receive as much as 126 mg of F during dialysis [16] which might be sufficient to result in enough F-conjugated proteins to serve as the antigenic component but a sufficient amount of IgG antibody would be required simultaneously to result in toxic antigen-antibody complexes. Patients with such IgG antibody levels may exist and the techniques described here may be used for analysis if there is clinical suspicion of immune complex disease. Only minute (ng) quantities of IgE antibody are required to result in an allergic reaction. IgE antibodies in sufficient amount for such reactions were found in 2 hemodialysis patients with no clinical signs of such a reaction. The possibility of such reactions deserves further study.

The IgG or IgE antibodies may be persistent or variable. Patients using F-sterilized dialyzers may be exposed to variable amounts of F which could either stimulate a persisting immune response or neutralize antibody after conjugation with HSA.

The immunologic studies on Serum 1 (fig. 3) indicate that in this subject the IgG antibody against F-HSA is directed against an antigenic grouping composed of F and altered configurations or new antigenic determinants on the HSA molecule. This was similarly defined for reactions of human antibodies against new antigenic determinants composed of trimellitic anhydride and HSA [12] and suggested many years ago for F rabbit serum proteins using the term neospecificity [13].

Following completion of analysis of studies of anti F-HSA antibodies in hemodialysis patients, we attempted to demonstrate antibodies in 4 subjects with acute respiratory symptoms to F, 3 of whom had been reported [3, 4]. The 2 subjects with acute rhinitis due to F exposure had no detectable antibodies. By contrast, the 2 nurses who were reported to have F-induced asthma and positive bronchial challenges had IgE antibody against F-HSA (S 21 and S 22 in fig. 7 and table I) [17]. Of note, however, is that these sera were also reactive to HSA alone. While a major consideration would be that the IgE antibody against both F-HSA and HSA is coincidental, studies by other investigators and our animal studies [8] suggest an alternate explanation. *Horsfall* [13] showed that some rabbits made antibodies against F-rabbit serum and against homologous rabbit serum proteins. *Onica* et al. [18] showed that glutaraldehyde-treated serum albumin was altered in a manner similar to albumin 'aged' in vitro. We showed [8] that some dogs immunized with F-DSA also reacted with DSA. Further, dogs receiving intravenous F may produce antibody against F-DSA or against DSA. It is thus postulated by *Onica* et al. [18] and suggested by the work of *Horsfall* [13] and us [8] that F modifies albumin in a manner similar to in vitro aging and antibody against F-modified albumin reacts with similar determinants on albumin after in vitro storage. In support of the selective action of F on HSA, antibodies to ethylene oxide-conjugated HSA [11] had antibody to unconjugated HSA and only 1 serum with anti trimellityl-HSA had a low titer to HSA. Further, serologic studies of 215 patients receiving multiple injections of HSA used as a stabilizer in allergenic extract showed no antibody response to HSA in any patient [19]. The antibody responses to F-HSA and HSA must be considered of potential biologic significance in subjects with F-induced symptoms.

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