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A Monoclonal Antibody Recognizes Structural Variation in Cystic Fibrosis α_2-Macroglobulin

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Summary

α_2-Macroglobulin (α_2M) is a major plasma protease inhibitor that has been studied because of its suggested role in the pathology of cystic fibrosis (CF). A panel of monoclonal antibodies specific for human α_2M were produced and screened for their ability to bind to a number of human α_2M samples. We have used these antibodies to characterize individual antigenic sites in this protein. α_2M was purified from plasma by polyethylene glycol precipitation followed by zinc chelate chromatography. A total of 23 α_2M samples in the native configuration, as well as the nucleophile-treated configuration, were screened by the panel of 18 monoclonal antibodies in an enzyme-linked immunosorbent assay procedure. Five of the samples tested were from individuals with cystic fibrosis. α_2M from family members of two of these patients was subsequently tested for reactivity with the monoclonal antibodies. One antibody, SAM94, exhibited a significant difference in binding to α_2M obtained from CF patients as compared with control individuals. This difference was particularly apparent in the binding of SAM94 to the nucleophile-treated CF α_2M; SAM94 showed significantly reduced binding to four of five unrelated CF individuals ($p < 0.005$) and three of four cystic fibrosis obligate heterozygotes ($p < 0.005$).

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Abbreviations

α_2M, α_2-macroglobulin
CF, cystic fibrosis
ELISA, enzyme-linked immunosorbent assay
PEG, polyethylene glycol
PBS, phosphate-buffered saline
PMSF, phenylmethylsulfonyl fluoride
SBTI, soybean trypsin inhibitor
TEMED, *N,N,N′,N′*-tetramethylethylenediamine

α_2M is a plasma glycoprotein that functions in the inhibition and elimination of proteases from the plasma (16). The molecule, composed of four subunit chains of 185,000 molecular weight (11), binds irreversibly to the active form of proteases, changing the conformation of α_2M and thereby trapping the enzyme (2). A similar conformational change can be mimicked by treatment

of α_2M with nucleophilic compounds such as methylamine (27). These altered molecules are cleared from the circulation in the same manner as the protease-α_2M complexes (12). The modified form shows a greater electrophoretic mobility on polyacrylamide gels than the native form and is often termed "fast α_2M."

Cystic fibrosis has been studied for many years although no biochemical or structural defect has been established (28). The probable involvement of α_2M in the pathology of cystic fibrosis has been suggested by several groups based upon their work on isoelectric focusing of serum samples (29) and immunological and biochemical examination of protease-α_2M complexes (22–25). However, recent reports have failed to confirm differences in structure and protease binding capabilities of cystic fibrosis and normal α_2M (5, 7, 8, 19).

Since the report of continuously growing cell lines producing homogeneous antibodies in 1975 (14), these monoclonal antibodies have provided a way to study the structure of proteins that was not possible with conventional antisera. Single determinants on a molecule can be examined with these reagents because of their unique specificities. We have produced monoclonal antibodies to human α_2M (9) and have used these antibodies to examine α_2M purified from plasma samples. In this report, we discuss our findings using a panel of anti-human α_2M monoclonal antibodies in an enzyme-linked immunoassay to examine a number of purified human plasma α_2M samples.

METHODS

Materials and antisera. All chemicals used were reagent grade unless otherwise indicated. Acrylamide, bis-acrylamide, and TEMED were obtained from Bio-Rad Laboratories, Richmond, CA. PEG 1000 used for fusions was from J.T. Baker Chemical Co, Phillipsburg, NJ. Bovine serum albumin determined to be peroxidase free) was from Calbiochem, San Diego, CA. Purified human immunoglobulin was obtained from Cappel Laboratories, Cochranville, PA. Zinc chelate-Sepharose 4B resin was kindly provided by Dr. J. Kaplan, University of Utah, Salt Lake City, UT. PMSF, o-phenylene diamine, polyethylene glycol 6000, SBTI (Type 1S), Tween 80 and all other chemicals not mentioned above were obtained from Sigma Chemical Co, St. Louis, MO.

Polystyrene 96-well microtiter plates (Immunlon) were from Dynatech Laboratories, Alexandria, VA.

Peroxidase conjugated sheep anti-mouse immunoglobulin and rabbit anti-rat immunoglobulin were obtained from Cappel Laboratories.

Plasma. Fresh plasma from individuals with cystic fibrosis and heterozygotes was obtained by Dr. D. Holsclaw from patients and family members seen at the Cystic Fibrosis Clinic at Hahnemann Hospital and Medical College, Philadelphia, PA. Normal plasma was supplied by the Tissue Typing Laboratory of the Hospital of the University of Pennsylvania and the Blood Bank of Children's Hospital of Philadelphia. Blood was collected in vacutainers containing 15% EDTA. Plasma was separated by centrifugation, and PMSF was added to a final concentration of 10 mM. Sodium azide was added to 0.1%. The preparation was frozen at −70°C.

Isolation and purification of α_2M. α_2M was purified from plasma by polyethylene glycol precipitation and zinc chelate chromatography according to the methods described by Barrett *et al.* (1) and Kurecki *et al.* (15). Plasma samples (5–50 ml), to which SBTI was added (0.1 mg/ml), were rapidly thawed and 0.28 volumes of 25% (W/V) PEG 6000, pH 6.5 were mixed with each sample. The solution was kept at room temperature for 30 min then centrifuged at 4°C at 10,000 × *g* for 30 min. 0.72 volumes of 25% PEG was added to the supernatant and allowed to sit at room temperature for 30 min. The plasma-PEG solution was centrifuged as described above. The precipitate was dissolved in 20 mM sodium phosphate, pH 6.4 and dialyzed overnight against distilled water.

The dialysate was applied to a 1.0 × 11 cm column of Sepharose 4B which had been treated with zinc chloride. The column was washed with several column volumes of 0.2 M sodium phosphate, 0.15 M NaCl, pH 6.0, and the α_2M eluted with 0.02 M sodium cacodylate, 0.15 M NaCl, pH 5.0 (15). The optical density of each fraction was determined at 280 nm and samples containing sufficient quantities of protein were pooled. Concentration of α_2M was calculated using an extinction coefficient of 8.93 (31). Regeneration of the column was accomplished by washing with 0.05 M EDTA, 0.5 M NaCl, pH 7.0 followed by equilibration of the resin with zinc chloride (3 mg/ml, pH 6.0) and washed with 0.25 M sodium acetate, 0.15 M NaCl, pH 5.0. Pooled samples of α_2M were dialyzed against PBS, pH 7.2 and stored in glass tubes at 4°C with 0.1% sodium azide.

Treatment of α_2M with methylamine. Purified α_2M that was modified by reaction with methylamine was initially dialyzed against several changes of 0.05 M Tris HCl, pH 8.0 for 48 h or until the pH of the α_2M solution was 8.0. Methylamine was added to a final concentration of 200 mM and the solution incubated for 2 h at 37°C. The modified α_2M was stored at 4°C with 0.1% sodium azide.

Electrophoresis of α_2M forms. α_2M purified by zinc chelate chromatography and samples modified by treatment with methylamine were examined by polyacrylamide gel electrophoresis. Samples dissolved in sample buffer containing 0.16 M Tris, pH 8.8, 10.7% glycerol and 0.01% bromphenol blue were applied to a 5–15% discontinuous nondenaturing gradient gel as described by Zais and Roberts (31) and run at 200 V for 3 h. The gel was stained for protein with Coomassie brilliant blue.

Production of anti-human α_2M hybridomas. Hybridomas were produced from the fusion of spleen cells from mice and rats immunized with purified human α_2M and Sp2/0-Ag14, a non-secreting mouse myeloma (26). The method of immunization, maintenance of cells, and specificity of antibodies are described elsewhere (9, 13).

Enzyme-linked immunosorbent assay. The source of monoclonal antibodies was cell culture supernatant removed from cells that were in mid-log phase. Supernatant was stored at 4°C with 0.1% sodium azide. Antisera used as the positive control in these assays were obtained from bleeding of the immunized animals at the time of sacrifice prior to the removal of the spleen for fusion with the myeloma cells. The ELISA procedure was performed as described previously (9) although in this case purified α_2M was used.

Purified α_2M diluted in 0.05 sodium carbonate, pH 9.8 was added to the wells of a distilled water rinsed 96-well polystyrene plate (0.1 μg α_2M in 50 μl/well). The protein was incubated for 18 h at 4°C and then rinsed twice with a solution of 0.05% Tween 80 in PBS, pH 7.2 (TwPBS). Monoclonal antibodies were added and incubated for 2 h at room temperature. Following 2–4 washes of TwPBS, peroxidase conjugated antisera, mixed with an equal volume of human immunoglobulin and preincubated for 18 h at 4°C, was diluted in TwPBS containing an additional 2% NaCl, 0.05 M EDTA and 0.1% bovine serum albumin (peroxidase free), added to each well of the polystyrene plate and incubated for 2 h. Following the incubation, the plates were washed 3–5 times with TwPBS. 150 μl of substrate solution (5.5 mM o-phenylene diamine and 12 mM hydrogen peroxide in 0.1 M sodium citrate buffer, pH 4.5) was added to each well of the plate and incubated for 15 min at room temperature. The colorimetric reaction was stopped by the addition of 25 μl of 0.1 M sodium fluoride and the optical densities determined at 450 nm using a Multiscan spectrophotometer (Flow Laboratories, McLean, VA).

RESULTS

Preparation of α_2M for enzyme-linked immunosorbent assay. Fresh plasma samples from presumably normal volunteers and patients with cystic fibrosis (mild to moderate severity of disease)

were the sources of α_2M used in these experiments. The method for purification using polyethylene glycol precipitation followed by passage of the α_2M containing fraction over a zinc chelate complexed Sepharose column provided us with α_2M in the native form. The elution profile from the zinc chelate column of polyethylene glycol purified α_2M is shown in Figure 1. The first peak consists of unbound plasma proteins whereas the second peak is column purified α_2M which was pooled and dialyzed against phosphate buffered saline. The use of fresh plasma yields product which has been shown to contain α_2M in primarily the native form. The conversion of native, electrophoretically "slow" α_2M by treatment with 200 mM methylamine to the electrophoretic "fast" form is shown to be nearly complete as seen in Figure 2. The electrophoresis of samples of untreated and chemically modified α_2M on a 5-15% discontinuous gradient polyacrylamide gel demonstrates that a high percentage of α_2M isolated by the PEG precipitation and zinc chelate chromatography is in the electrophoretically slow form (Fig. 2, lanes 1, 3, 5, 7). In lanes 2, 4, and 6, one notices an increase in electrophoretic mobility after treatment with methylamine of the same α_2M sample from lanes 3, 5, and 7. The conversion to the fast form is nearly complete.

Assay using monoclonal antibodies to analyze human α_2M. Monoclonal antibodies have been produced against human α-2-macroglobulin as described elsewhere (9). These rat and mouse monoclonal antibodies have been shown to react with α_2M in both the native and methylamine-treated forms. A panel of 18 monoclonal antibodies reacting with human α_2M were selected to analyze the structure of a number of α_2M samples. α_2M from the purification described above was quantitated by determination of the absorbance of the solution at 280 nm.

The levels of reaction of the monoclonal antibodies with the α_2M samples were determined by the use of an enzyme-linked immunoassay to quantitate the antibody binding to a given concentration of the purified antigen. Plasma α_2M in both the native and methylamine-treated forms (slow and fast forms, respectively) were screened with the panel of monoclonal antibodies which had been selected on the basis of stability of expression and affinity of antibody. For each α_2M sample purified, 0.1 μg of α_2M was applied per well of a polystyrene plate; duplicate determinations for each antibody were performed on at least three independent occasions. Absorbance values were normalized for each antibody in the individual assays according to the equation $V = (A - B)/(C - B)$ where A is the absorbance value of the monoclonal antibody binding to sample n, B is the absorbance value of nonspecific binding of Sp2/0-Ag14 supernatant to sample n, and C is the absorbance value of the standard anti-human α_2M sera obtained from the immunized animals. These normalized values are termed binding ratios. The levels of binding of standard antisera to individual α_2M samples were equivalent.

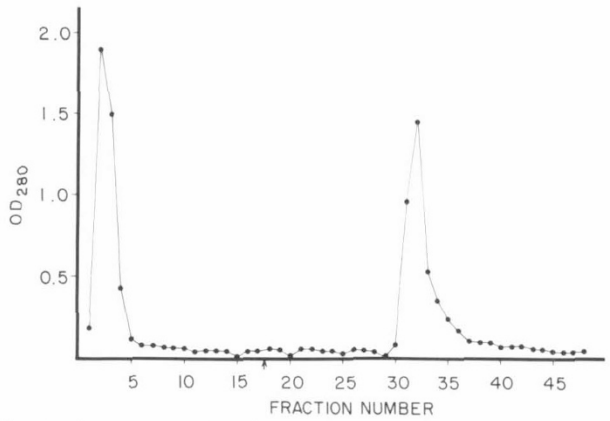

Fig. 1. Chromatography of α_2M on zinc chelate column. Dialyzed α_2M purified from 6 ml of plasma was charged on a (1.0 × 11 cm) zinc chelate-Sepharose column equilibrated with 0.02 M sodium phosphate, 0.15 M sodium chloride, pH 6.0, at a flow rate of 100 ml/h. Elution was begun with the same buffer at 50 ml/h and 9.2 ml fractions were collected. At fraction 18, the buffer was changed to 0.02 M sodium cacodylate, 0.15 M sodium chloride, pH 5.0 and 0.9 ml fractions were collected. Fractions 31–34 were pooled, dialyzed against PBS and stored with azide.

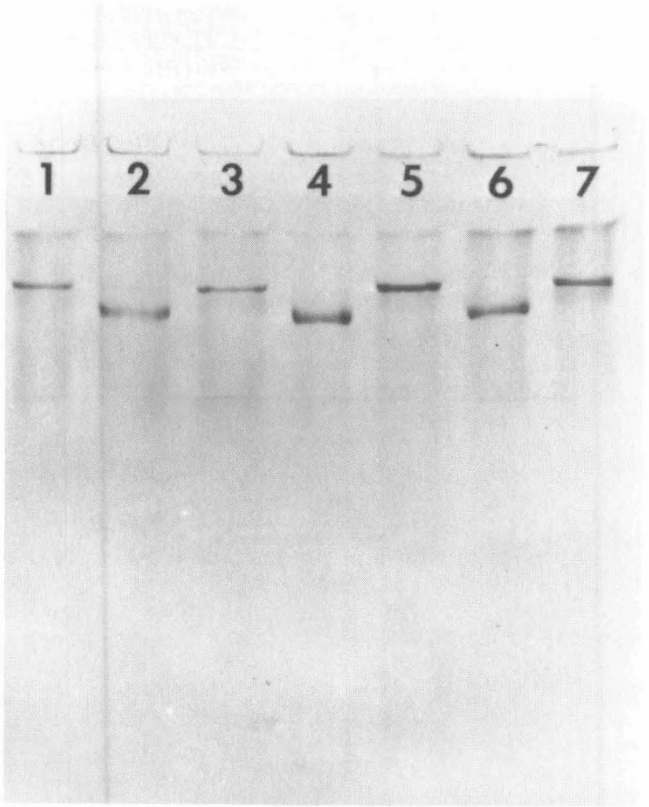

Fig. 2. Changes in electrophoretic mobility of α_2M resulting from treatment with methylamine. Human α_2M in 0.05 M Tris HCl, pH 8.0 was treated with 200 mM methylamine for 1 h at 37°C. Samples of human α_2M were examined by acrylamide gradient pore gel electrophoresis. The gel was stained with Coomassie brilliant blue R-250. Lane 1 is Sample 12 α_2M untreated; lane 2 Sample 11 α_2M treated with methylamine; lane 3 Sample 11 untreated; lane 4 Sample 10 treated; lane 5 Sample 10 untreated; lane 6 Sample 9 treated, and lane 7 Sample 9 untreated.

Reactivity patterns. We show in this paper the results of the binding of three antibodies to the group of α_2M samples. The two antibodies SAR87 and SAM167 were chosen for discussion here on the basis of their reactivity patterns and are representative of all except one of the 18 antibodies with respect to the reactivities towards cystic fibrosis and control α_2M samples. SAM94 exhibits a reactivity pattern unlike the other antibodies. The binding ratios for each of the three antibodies were calculated for duplicate determinations in three independent assays. The mean and standard deviation of the binding ratios for each antibody and each α_2M sample (both forms) were calculated and are listed in Table 1. Individuals 4, 5, 18, 19, and 20 are unrelated individuals with cystic fibrosis. Plasma α_2M of individuals from the families of individuals 4 and 19 were also tested. The pedigrees of these two families are shown in Figure 3. α_2M samples 8 and 11 are from the same individual although blood was drawn at different times and processed separately. The same situation is true for samples 14 and 15. The reproducibility of the purification procedure, as well as the enzyme-linked immunoassay, is demonstrated by comparison of the binding ratios given in Table 1 for these α_2M samples.

SAR87 is a rat monoclonal antibody which reacts similarly with both the slow form and fast form of α_2M (Table 1; Fig. 4).

Table 1. *Mean binding ratios for the reactions of three monoclonal antibodies (SAR87, SAM94, SAM167) to human α_2M samples in the native and chemically treated forms**

Genetic status†	Sample *n*	SAR87		SAM94		SAM167	
		Slow	Fast	Slow	Fast	Slow	Fast
N	2	0.175 ± 0.069	0.194 ± 0.083	1.707 ± 0.035	1.331 ± 0.033	0.055 ± 0.019	0.060 ± 0.032
N	3	0.264 ± 0.085	0.295 ± 0.035	1.156 ± 0.122	1.154 ± 0.128	0.073 ± 0.012	0.120 ± 0.033
N	6	0.203 ± 0.036	0.214 ± 0.053	1.124 ± 0.180	0.894 ± 0.072	0.134 ± 0.023	0.163 ± 0.031
N	7	0.198 ± 0.040	0.170 ± 0.027	0.869 ± 0.103	0.903 ± 0.068	0.143 ± 0.036	0.182 ± 0.043
N	8§	0.178 ± 0.057	0.163 ± 0.040	0.942 ± 0.070	0.818 ± 0.049	0.184 ± 0.049	0.187 ± 0.027
N	9	0.200 ± 0.072	0.204 ± 0.048	1.036 ± 0.104	0.960 ± 0.029	0.193 ± 0.044	0.260 ± 0.059
N	10	0.209 ± 0.021	0.157 ± 0.019	0.923 ± 0.044	0.853 ± 0.093	0.324 ± 0.042	0.339 ± 0.028
N	11§	0.144 ± 0.030	0.116 ± 0.022	1.008 ± 0.068	0.914 ± 0.054	0.213 ± 0.025	0.158 ± 0.048
N	12	0.245 ± 0.038	0.102 ± 0.056	1.077 ± 0.152	0.912 ± 0.094	0.107 ± 0.033	0.097 ± 0.024
N	13	0.139 ± 0.036	0.116 ± 0.033	1.185 ± 0.205	0.880 ± 0.120	0.174 ± 0.040	0.141 ± 0.052
N	14¶	0.213 ± 0.029	0.113 ± 0.056	0.902 ± 0.057	0.824 ± 0.150	0.261 ± 0.101	0.174 ± 0.053
N	15¶	0.182 ± 0.050	0.144 ± 0.029	0.958 ± 0.236	0.781 ± 0.074	0.294 ± 0.078	0.279 ± 0.028
N	16	0.264 ± 0.055	0.156 ± 0.039	0.896 ± 0.119	0.789 ± 0.092	0.287 ± 0.078	0.339 ± 0.034
N	17	0.138 ± 0.053	0.170 ± 0.060	0.904 ± 0.063	0.908 ± 0.094	0.164 ± 0.031	0.195 ± 0.017
CF	4	0.165 ± 0.075	0.252 ± 0.033	0.489 ± 0.056	0.313 ± 0.018	0.402 ± 0.028	0.576 ± 0.175
CF	5	0.236 ± 0.013	0.130 ± 0.031	1.097 ± 0.031	0.676 ± 0.069	0.205 ± 0.072	0.195 ± 0.034
CF	18	0.172 ± 0.023	0.155 ± 0.054	0.818 ± 0.093	0.851 ± 0.067	0.174 ± 0.030	0.199 ± 0.020
CF	19	0.289 ± 0.045	0.192 ± 0.016	0.715 ± 0.118	0.601 ± 0.066	0.202 ± 0.047	0.280 ± 0.032
CF	20	0.129 ± 0.048	0.137 ± 0.017	0.646 ± 0.099	0.439 ± 0.048	0.370 ± 0.032	0.400 ± 0.029
CF	22	0.226 ± 0.042	0.245 ± 0.027	0.814 ± 0.039	0.636 ± 0.030	0.133 ± 0.030	0.163 ± 0.022
CF	26	0.364 ± 0.078	0.338 ± 0.080	0.967 ± 0.128	0.714 ± 0.078	0.136 ± 0.049	0.155 ± 0.022
CFH	21	0.391 ± 0.034	0.329 ± 0.043	0.963 ± 0.024	0.796 ± 0.019	0.275 ± 0.017	0.260 ± 0.033
?‡	23	0.211 ± 0.068	0.176 ± 0.044	0.719 ± 0.092	0.603 ± 0.092	0.195 ± 0.031	0.171 ± 0.036
CFH	24	0.416 ± 0.064	0.291 ± 0.063	0.712 ± 0.065	0.466 ± 0.038	0.380 ± 0.060	0.380 ± 0.029
?	25	0.422 ± 0.093	0.338 ± 0.100	1.026 ± 0.080	0.946 ± 0.078	0.158 ± 0.046	0.156 ± 0.028
CFH	27	0.440 ± 0.057	0.366 ± 0.096	0.698 ± 0.151	0.563 ± 0.205	0.301 ± 0.121	0.299 ± 0.046
CFH	28	0.299 ± 0.106	0.229 ± 0.018	0.577 ± 0.121	0.557 ± 0.039	0.248 ± 0.079	0.230 ± 0.022

* Values shown are mean binding ratio ± SD.

† N, normal individual; CFH, cystic fibrosis obligate heterozygote.

‡ ?, unaffected sibling of CF patient, genetic status unconfirmed.

§ 8, 11, same individual, blood drawn at different times.

¶ 14, 15, same individual, blood drawn at different times.

Fig. 3. Pedigrees of families I and II. Solid figures represent individuals with CF; half-filled figures represent obligate heterozygotes; figures with question marks indicate nonobligate heterozygotes but possibly carriers of the gene.

Antibody SAM167 is a mouse monoclonal antibody that also reacts similarly with both forms of α_2M from the same individual although the binding ratios do not overlap as much as with SAR87. Antibody SAM94, although reacting with both forms, shows a very different pattern of binding to particular samples. SAM94 and SAM167 are antibodies of the IgG_1 isotype as determined by immunoprecipitation with class specific antisera.

The mean binding ratios for these three antibodies are shown in graphical form in Figure 4. In addition, the genetic status of the individuals is noted by a solid square for those individuals with CF, half-filled squares for CF family members, and open squares for control individuals. Note the distribution of solid and half-filled squares. SAM94 (Fig. 4, panel C) shows a clustering of these samples at lower binding ratios in both forms of α_2M. With antibodies SAR87 and SAM167 (panels A and B, respectively), there is a random distribution of binding ratios from CF homozygotes and family members among the control individuals. Only with SAM94 is there a clustering of mean binding ratios from CF homozygotes and family members in both the slow and fast forms of α_2M. When the standard deviations are considered, the binding of the samples from CF homozygotes and certain family members are clearly distinguished from the remainder of the samples. Figure 5, a replotting of the data in Figure 4, panel C of the antibody reaction with the fast form (methylamine-treated) of α_2M, more clearly distinguishes the differences in antibody reactivity to individual samples of α_2M. The unaffected obligate heterozygotes are marked by an asterisk.

The significance of the difference between the mean binding ratio for normal α_2M samples (methylamine-treated) and the mean binding ratio for CF (α_2M methylamine-treated) samples with antibody SAM94 was determined by a Student t test. The difference between the 12 normal samples (0.938 ± 0.154; mean ± SD) and the 5 unrelated CF samples (0.576 ± 0.209) is statistically highly significant ($p < 0.005$)($t = 4.02$). Also, comparison of the means of the four obligate heterozygote samples (0.595 ± 0.141) is significantly different from that of the normal samples at the $p < 0.005$ level of significance ($t = 3.94$). However, a t test comparing CF and obligate heterozygotes binding ratios does not show any significant difference between these two groups ($t = -0.155$).

Although the difference in the binding ratios of antibody SAM94 to CF and normal α_2M is based only on a limited number of samples, the genetic status of unaffected individuals who are not obligate heterozygotes can be inferred using the binding ratios. In family I, unaffected individual 23 has a 66% probability of carrying the gene for cystic fibrosis. The binding ratio of SAM94 to methylamine-treated α_2M from this individual is in the range observed for CF obligate heterozygotes and homozygotes. On the other hand, methylamine-treated α_2M from individual 25 of family II when reacted with SAM94 gives

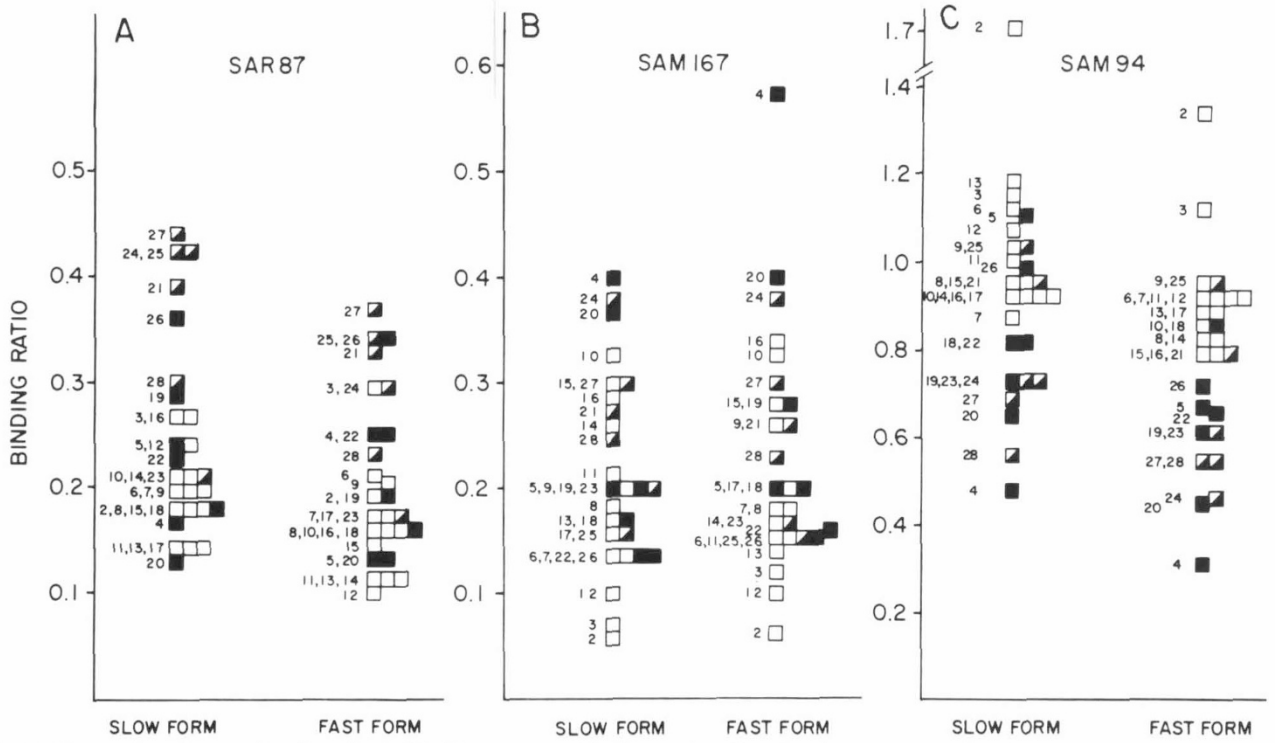

Fig. 4. Composite of monoclonal antibody binding patterns. Binding ratios of antibodies to slow native (untreated) and fast (methylamine-treated) forms of α_2M from the plasma samples described in Table 1 are shown in this figure. Individuals of different genetic background are highlighted. Each square represents an individual; open squares, control individuals; solid squares, CF homozygotes and half-filled squares, CF family members whether obligate heterozygotes or not.

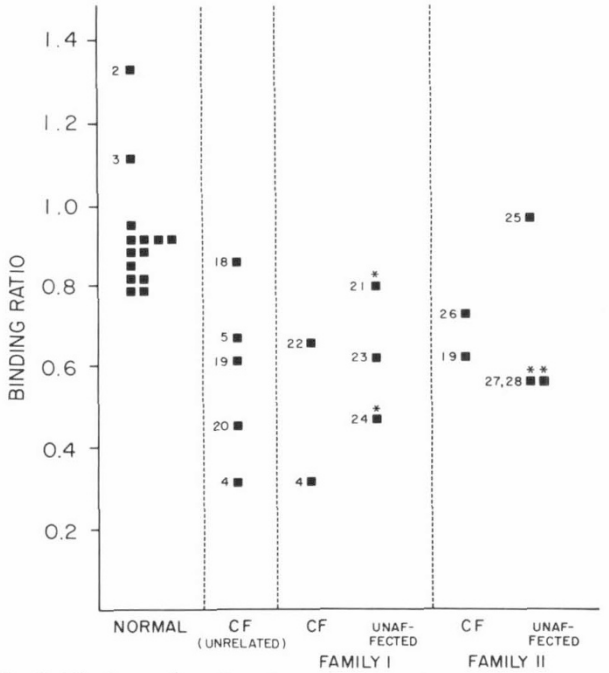

Fig. 5. Binding ratios of methylamine-treated α_2M samples of families with the antibody SAM94. Graph shows the distribution of binding ratios of α_2M samples when divided into categories based upon the genetic status. Family members from the two families tested are divided into those members affected with CF and those unaffected. Each square represents one individual; numbers to the left of the squares correspond to the sample number. Obligate heterozygotes are distinguished by an asterisk.

a binding ratio similar to those values calculated for normal α_2M samples.

The variability of binding ratios to methylamine-treated cystic fibrosis α_2M that we observe with antibody SAM94 may be accounted for by the heterogeneity in the disease (Table 1; Fig. 5). Examination of the five α_2M samples from unrelated individuals with cystic fibrosis by binding of SAM94 shows that one gives a binding ratio similar to that of control individuals. In addition, we see a range of binding ratios for the remaining four unrelated samples. Medical treatment and age of the patients appear to have no effect on the results obtained. Since the frequency of CF heterozygotes in the general population is approximately 0.05, we expect in our presumably normal sample of 12 controls $0.05 \times 12 = 0.6$, or about one individual carries the gene for cystic fibrosis. However, none of the normal α_2M samples tested give binding ratios as low as the mean binding ratio of the CF samples.

DISCUSSION

The examination of purified human α_2M with monoclonal anti-α_2M antibodies has resulted in an interesting observation. When normal and cystic fibrosis α_2M are reacted with monoclonal antibody SAM94, a lower level of binding is seen with the CF samples. When examining the nucleophile-treated form of α_2M with antibody SAM94 under the same conditions, the decreased level of reactivity is more apparent. This decrease in antibody reactivity to CF α_2M in both the native form as well as with the nucleophile-treated protein is not observed when other anti-α_2M monoclonal antibodies are used.

It has been hypothesized that structural alteration in CF plasma α_2M is responsible for the presence of protease-α_2M complexes and abnormal peptides. However, several reports do not support the idea that there is an abnormality in CF α_2M. The interaction of proteases with α_2M did not demonstrate any difference with respect to protease binding ability in CF α_2M nor were the α_2-macroglobulins distinguishable by isoelectric focusing (18, 20, 21). The examination of the cleavage of subunits of α_2M when reacted with trypsin showed similar electrophoretic patterns when purified α_2M was passed over a column of trypsin immobilized on Sepharose (6). Recent reports indicate protease binding and structure of α_2M in CF and controls are similar (5,

7, 8, 19). The use of monoclonal antibodies, however, allows the examination of a single antigenic determinant which if altered may have no detectable effect on the capacity to bind proteases or affect the mobility of the protein in electrophoresis. Monoclonal antibodies specific for the nucleophile-treated form of α_2M have been identified (17).

Our antibody, SAM94, appears to detect an alteration in CF and CF heterozygote α_2M; this difference is enhanced when the protein is treated with methylamine. We hypothesize that this antibody identifies a determinant which has: 1) a decrease in frequency on the CF α_2M molecule, or 2) an alteration in the antigenic site thereby causing reduced affinity of the antibody for this site, or 3) a modification in the conformation of nucleophile-treated CF α_2M. A report by Ben-Yoseph and colleagues (3, 4) indicates they have identified a decrease in glycosylation of CF α_2M. Preliminary experiments of our own suggest that the antibody may be recognizing a determinant of carbohydrate origin.

A defect in the autoregulation of the monocytes and macrophages has been postulated by Wilson and Fudenberg (30). They report that α_2M receptors on CF monocytes-macrophages are not abnormal, but that the defect resides in the α_2M and subsequently results in the accumulation of CF factors. Our examination using SAM94 to study methylamine-treated α_2M suggests a structural difference is present in the α_2M from CF patients and CF obligate heterozygotes. Analysis of the relationship between the antigenic site of the molecule detected by SAM94 and the portion of the α_2M interacting with the receptor may prove informative.

The CF patients and families that have been screened clearly indicate that antibody SAM94 is identifying a determinant present on the α_2M of certain individuals. The differential binding to CF samples could possibly be due to the presence of a polymorphism unrealted to cystic fibrosis. Further screening of CF individuals and family members must be undertaken to test the reliability of the antibody for identifying those individuals carrying the gene for cystic fibrosis. The data do confirm the idea that monoclonal antibodies are useful for detecting subtle structural variations in macromolecules and that they can in fact be used to detect differences not easily identified by other methods.

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