JCI The Journal of Clinical Investigation

Replacement therapy of alpha 1-antitrypsin deficiency. Reversal of protease-antiprotease imbalance within the alveolar structures of PiZ subjects.

J E Gadek, … , P V Holland, R G Crystal

J Clin Invest. 1981;68(5):1158-1165. https://doi.org/10.1172/JCI110360.

Research Article

The emphysema associated with the inherited serum deficiency of alpha 1-antitrypsin appears to result from an imbalance between neutrophil elastase and its major inhibitor within the alveolar structures. In the present study we assessed the feasibility of reversing this biochemical defect within the lung via parenteral replacement therapy with an alpha 1-antitrypsin concentrate of normal plasma. A 20--40% polyethylene glycol precipitate of pooled human donor plasma was used to obtain an enriched alpha 1-antitrypsin concentrate devoid of hepatitis B antigen and immunoglobulins. Using this material, five individuals with severe serum alpha 1-antitrypsin deficiency (PiZ phenotype) and advanced emphysema received 4 g of alpha 1-antitrypsin intravenously at weekly intervals for four doses. During this period of weekly replacement therapy alpha 1-antitrypsin serum levels were maintained at greater than or equal to 70 mg/dl, the level likely required for effective antielastase protection of the lung. In addition, assessment of lower respiratory tract antielastase activity by bronchoalveolar lavage demonstrated that parenteral replacement of alpha 1-antitrypsin resulted in establishment of effective antielastase activity within the alveolar structures. There were no untoward side effects consequent to this approach to the replacement therapy of alpha 1-antitrypsin. These results demonstrate that the parenteral replacement of alpha 1-antitrypsin provides a means of obtaining elastase-antielastase balance within the lung of individuals with this serum protease inhibitor deficiency.

Replacement Therapy of Alpha 1-Antitrypsin Deficiency

REVERSAL OF PROTEASE-ANTIPROTEASE IMBALANCE WITHIN THE ALVEOLAR STRUCTURES OF PiZ SUBJECTS

JAMES E. GADEK, HARVEY G. KLEIN, PAUL V. HOLLAND, and RONALD G. CRYSTAL,
Pulmonary Branch, National Heart, Lung, and Blood Institute and Blood Bank, Clinical Center, National Institutes of Health, Bethesda, Maryland 20205

ABSTRACT The emphysema associated with the inherited serum deficiency of α1-antitrypsin appears to result from an imbalance between neutrophil elastase and its major inhibitor within the alveolar structures. In the present study we assessed the feasibility of reversing this biochemical defect within the lung via parenteral replacement therapy with an α1-antitrypsin concentrate of normal plasma. A 20–40% polyethylene glycol precipitate of pooled human donor plasma was used to obtain an enriched α1-antitrypsin concentrate devoid of hepatitis B antigen and immunoglobulins. Using this material, five individuals with severe serum α1-antitrypsin deficiency (PiZ phenotype) and advanced emphysema received 4 g of α1-antitrypsin intravenously at weekly intervals for four doses. During this period of weekly replacement therapy α1-antitrypsin serum levels were maintained at ≥70 mg/dl, the level likely required for effective antielastase protection of the lung. In addition, assessment of lower respiratory tract antielastase activity by bronchoalveolar lavage demonstrated that parenteral replacement of α1-antitrypsin resulted in establishment of effective antielastase activity within the alveolar structures. There were no untoward side effects consequent to this approach to the replacement therapy of α1-antitrypsin. These results demonstrate that the parenteral replacement of α1-antitrypsin provides a means of obtaining elastase-antielastase balance within the lung of individuals with this serum protease inhibitor deficiency.

INTRODUCTION

α1-Antitrypsin deficiency is a chronic, usually fatal autosomal codominant disorder in which a low concentration of serum α1-antitrypsin is associated with progressive, panacinar emphysema (1–4). The discovery of this association, together with the demonstration that enzymes with elastolytic activity induced emphysema when instilled into the lower respiratory tract of animals (5, 6), have led to what is known as the "protease-antiprotease" theory of emphysema. This theory holds that in normal individuals, the alveolar structures of the lower respiratory tract are protected from proteases released by inflammatory cells by a normal antiprotease screen (7). In the context of α1-antitrypsin deficiency, therefore, the protease-antiprotease theory hypothesizes that the alveolar structures are unprotected, and thus are progressively distorted by proteases released by inflammatory cells present in the lower respiratory tract.

α1-Antitrypsin is a glycoprotein of 52,000 daltons produced in the hepatocyte and released into the circulation at a rate of 32 mg/kg per d (8, 9). In normal individuals (so-called PiM, for "protease inhibitor" type M), this results in a serum α1-antitrypsin level of 150–250 mg/dl. Direct evaluation of the antiprotease screen of PiM individuals has shown that α1-antitrypsin represents >95% of the antielastase screen of the lower respiratory tract (10).

In contrast, in the PiZ homozygote, the most common α1-antitrypsin phenotype associated with emphysema, a substitution of a lysine for a glutamic acid in the α1-antitrypsin polypeptide chain appears to account for an abnormality in intracellular processing resulting in decreased secretion of the protein (11). Thus, even though the PiZ α1-antitrypsin molecule is just as efficient an antiprotease as the PiM molecule (2), the reduced secretory rate of the PiZ protein results in a markedly decreased serum α1-antitrypsin level, usually in the range of 15–50 mg/dl (12). Direct evaluation of the antiprotease screen of the PiZ individual has shown that this serum concentration of α1-antitrypsin is insufficient to afford antielastase protection of the lower respiratory tract, making the alveolar structures of the

Received for publication 2 October 1980 and in revised form 14 July 1981.

TABLE I

Clinical and Physiological Description of the Study Population

Patient	α1-Antitrypsin phenotype	Age	Sex	Duration of symptoms	Smoking history	Serum α1-antitrypsin	Vital capacity (% predicted)	Total lung capacity (% predicted)	RV/TLC‡ (% observed)	FEV_1§ (% predicted)	MMEF‖ (% predicted)	D_{LCO}¶ (% predicted)
		yr		*yr*	*packs/yr*	*mg/dl*						
1	PiZ	38	M	5	30	32	78	103	54	23	12	38
2	PiZ	37	M	4	20	42	51	115	62	39	10	35
3	PiZ	37	M	6	20	35	49	112	67	23	10	35
4	PiZ	66	F	8	0	43	111	103	38	51	8	40
5	PiZ	42	M	6	30	37	60	110	62	21	12	39
		44±5*		6±1		38±3	70±10	109±4	57±8	31±7	10±1	37±3

* Mean±SEM.

‡ RV/TLC, residual volume/total lung capacity.

§ FEV_1, forced vital capacity in 1 s.

‖ MMEF, maximum midexpiratory flow rate (25–75% of vital capacity).

¶ D_{LCO}, diffusing capacity; single breath method.

PiZ homozygote vulnerable to unimpeded elastolytic attack (10).

Because α1-antitrypsin is the major antiprotease of the lower respiratory tract, and because PiZ homozygote α1-antitrypsin deficient individuals have insufficient α1-antitrypsin in their serum (and hence in their alveolar structures) to protect the lower respiratory tract, the most direct and logical therapeutic approach to this inherited lung disease would be parenteral replacement of this missing antiprotease. The present study was designed to evaluate the feasibility of this approach. We have developed a relatively rapid and simple method for partially purifying α1-antitrypsin from pooled human plasma. With the knowledge that the serum half-life of α1-antitrypsin is 5.4 d (8), we devised an infusion schedule that would allow once weekly administration of α1-antitrypsin to PiZ individuals, thus maintaining serum α1-antitrypsin levels at >35% of normal, the level thought to be the serum threshold for antiprotease protection of the lower respiratory tract.

Using such a treatment schedule, we infused α1-antitrypsin intravenously into five individuals each week for 4 wk. Importantly, direct evaluation of the antiproteases of the lower respiratory tract of these individuals demonstrated that following such infusions, significant amounts of α1-antitrypsin with full antielastase activity do diffuse through the lower respiratory tract, thus likely affording protection to the alveolar structures from proteolytic attack.

METHODS

Study population. Five individuals with the α1-antitrypsin phenotype PiZ were admitted to the Pulmonary Branch service of the Clinical Center of the National Institutes of Health to participate in the study. There were four males and one female with a mean age of 43±5 yr.[1] Four had been cigarette smokers but none were currently smoking. All had roentgenographic and physiologic evidence of severe emphysema (Table I). At the time of evaluation no patient had produced sputum for at least 24 mo.

Preparation of α-1 antitrypsin plasma fraction. Normal, pooled human plasma was obtained from healthy, volunteer blood donors by the National Institutes of Health Blood Bank. All plasma used in this study was free of hepatitis B antigen as determined by radioimmunoassay. Each weekly infusion, containing ~4.0 g of α1-antitrypsin, was prepared from 3.5–4.0 liters of pooled plasma. An enriched fraction of α1-antitrypsin was obtained from the pooled plasma by sequential precipitation with polyethylene glycol (Carbowax 4000; Fisher Scientific Co., Pittsburgh, Pa.). Polyethylene glycol was added to the plasma at 4°C with vigorous stirring to obtain a final polyethylene glycol concentration of 20%. Following centrifugation (5,000 *g*) at 4°C for 3 h, the precipitate was discarded and the 20% supernate was again treated with polyethylene glycol to achieve a final concentration of 40%. This material was maintained at 4°C for 3 h and then centrifuged at 5,000 *g* for 4 h. The supernate was then carefully decanted and discarded. The 20–40% polyethylene glycol plasma precipitate was washed 3 times with deionized water resuspended in sterile, 0.9% NaCl to a final volume that was ~25% of the starting plasma volume.

The enriched α1-antitrypsin plasma fraction was then sterilized by ultrafiltration (0.45 μm pore size, Millipore Corp., Bedford, Mass.) and then submitted for sterility and pyrogen testing. All material used in the study was sterile and devoid of pyrogenic material that could be detected using standard Limulus lysate or rabbit assays for pyrogen. The α1-antitrypsin preparation was stored at −20°C for no longer than 2 wk before its intravenous administration. A representative preparation contained 400 mg/dl of α1-antitrypsin and 7.5 g/dl of albumin. The total protein content was ~8.0 g/dl and the osmolarity of the preparation was 450 mosmol/liter. The α1-antitrypsin fraction was devoid of immunoglobulins G and M as well as α2-macroglobulin but retained ~80% of the α1-anti-

[1] All data are presented as mean±SEM.

trypsin present in the starting material. Functional titration of this α1-antitrypsin fraction demonstrated no significant reduction of specific inhibitory activity when assayed with purified neutrophil elastase (see below).

Study design. After a routine medical examination, including liver and renal function tests, base-line levels of serum α1-antitrypsin were measured. All participants were advised of the potential risks of the study and provided with a detailed explanation of the protocol design that had been approved by the National Institutes of Health Human Research Committee. In particular, subjects in this study were informed of the risk of transfusion hepatitis before consent was obtained. Each individual then received the enriched α1-antitrypsin preparation intravenously over a 6–8-h period at weekly intervals for 4 consecutive wk. Serum was obtained for antigenic and functional α1-antitrypsin determinations (see below) immediately following the α1-antitrypsin infusion and at daily intervals thereafter.

During the base-line period, and on days 10 and 24 (2 d after the second and fourth infusions, respectively), those patients with a forced vital capacity in one second (FEV_1) of >0.75 l underwent fiberoptic bronchoscopy with lavage to evaluate the antiprotease screen of their lower respiratory tract (12). Using previously described methods, the lavage material was collected, the cells pelleted, and the fluid evaluated for α1-antitrypsin levels and antielastase function (see below). As previously described, all measurements were related to lavage fluid albumin levels to afford comparison to serum (13).

At 2 wk and 6 mo following completion of the study, serum was obtained from each individual to evaluate the possibility of transmission of hepatitis B viral antigen during the course of the α1-antitrypsin infusion. The serum samples were also used to evaluate possible production of α1-antitrypsin antibodies resulting from the infusions (see below).

Quantification of amount, type, and form of the α1-antitrypsin in the lung and blood of the study population. Immunochemical determination of serum α1-antitrypsin in the PiZ individuals during the course of the 4 wk study was performed with a standard radial immunodiffusion method (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) (14). The results of these immunochemical determinations were then expressed as a percent of normal serum α1-antitrypsin levels (normal levels were defined using a pool of normal PiM sera whose α1-antitrypsin concentration was 200 mg/dl). The quantity of α1-antitrypsin recovered by bronchoalveolar lavage was determined by "rocket" immunoelectrophoresis using the IgG fraction of monospecific goat antiserum to human α1-antitrypsin (Atlantic Antibodies, Westbrook, Maine) (15). Serum and bronchoalveolar albumin levels were quantified by radial immunodiffusion (Calbiochem-Behring Corp.).

Pi phenotyping of the sera and lavage fluid of the study patients was performed by isoelectric focusing in polyacrylamide slab gels (LKB, Rockville, Md.; pH 4.5 to 6.0) (16).

The form of the α1-antitrypsin in the lung of the study patients receiving α1-antitrypsin infusions was evaluated by crossed immunoelectrophoresis of bronchoalveolar lavage fluid. This was performed by slicing the polyacrylamide focusing gel and placing the strip onto an agarose plate containing antibody to α1-antitrypsin and electrophoresing the sample at pH 8.2 (17). Representative sera from PiM and PiZ individuals were used as standards.

Evaluation of the antielastase protection to lung and blood afforded by α1-antitrypsin infusions. Functional α1-antitrypsin activity in serum and lower respiratory tract fluid was determined by measuring the capacity of a given sample to inhibit the action of 1.0 μg of purified neutrophil elastase (18, 19) on an insoluble [^{3}H]elastin substrate. Functional antielastase activity was expressed as the percent of total inhibition of 1.0 μg of neutrophil elastase achieved by each sample (20).

μg of neutrophil elastase achieved by each sample (19).

Evaluation of serum for α1-antitrypsin antibodies. Sera were obtained from each of the five PiZ patients 3–6 mo after completion of the α1-antitrypsin infusion. These samples were assayed for the presence of precipitating antibody directed against the normal α1-antitrypsin (M) protein by counterimmunoelectrophoresis in 1% agarose gels (0.2 M veronal acetate buffer, pH 8.2) (21).

RESULTS

Response of serum α1-antitrypsin levels to α1-antitrypsin infusions. Each of the five patients tolerated the four weekly infusions to the α1-antitrypsin concentrate without significant adverse effects. The alteration of serum α1-antitrypsin levels observed in two patients during the four weekly intravenous infusions of 4.0 g of α1-antitrypsin are representative of the results obtained in all five subjects (Fig. 1). In each patient, the peak of serum α1-antitrypsin antigenic activity obtained immediately after the first infusion represented four- to fivefold increments over base-line levels. The serum α1-antitrypsin levels then declined sharply for 2–3 d;

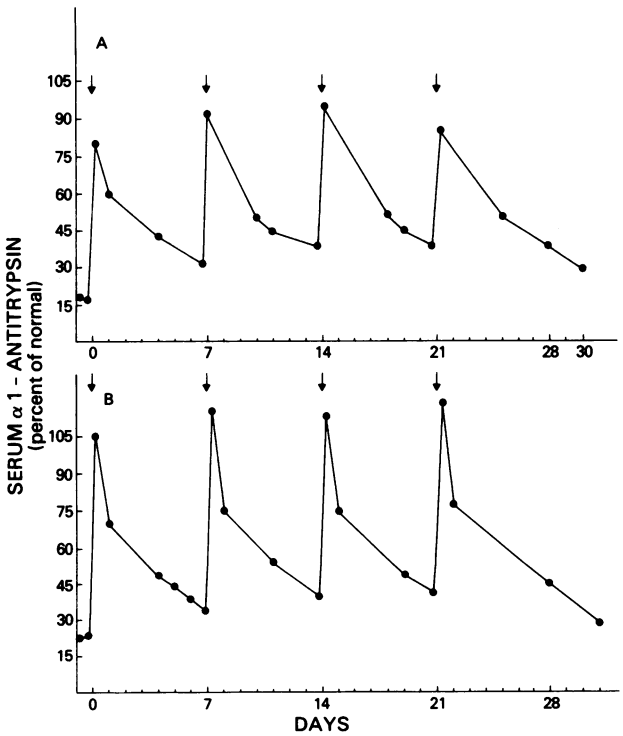

FIGURE 1 The response of serum α1-antitrypsin levels to the infusion of 4.0 g of α1-antitrypsin: (A) in patient 1; and (B) in patient 2. 4 g of α1-antitrypsin were infused intravenously at weekly intervals as shown by the arrows (↓). Serum values are expressed as a percentage of the level found in a pool of normal (PiM) donors.

this steep fall likely reflected redistribution of the administered α1-antitrypsin throughout the extracellular space (a volume estimated at 1.8 times the plasma volume) as well as catabolism of the infused α1-antitrypsin (8). A more linear portion of α1-antitrypsin clearance from the serum was noted from day 3 to 7. The serum levels on days 3 and 7 following the initial infusion represented increments of 2.5- and 1.5-fold over pretherapeutic levels, respectively. These data suggest that the fractional catabolic rate of the α1-antitrypsin administered intravenously was ~12%/d, in close agreement with the studies of α1-antitrypsin half-life carried out using radioiodinated α1-antitrypsin (8). This finding also suggests that the polyethylene glycol procedure used to prepare the plasma fraction in this study does not accelerate the clearance of α1-antitrypsin from the circulation.

As expected from the study design, the "trough" α1-antitrypsin level (day 7 after infusion) obtained just prior to the next α1-antitrypsin infusion increased during each successive week of the study. For example, the trough serum α1-antitrypsin levels obtained in patient 1 on successive weeks were 1.5, 1.65, 1.75, and 1.75 times the pretreatment level (Fig. 1A). This presumably reflects the fact that with a fractional catabolic rate of 12%/d, nearly 30% of the infused α1-antitrypsin remained within the extracellular compartment 7 d after administration.

As a group, the serum levels of α1-antitrypsin in all five patients (in response to each of the infusions) was similar (Table II). Each patient received four infusions at weekly intervals; for each of these infusions, the serum level achieved at day 2 after infusions was three- to fourfold that of the pretreatment level. Importantly, for each infusion, all patients maintained serum α1-antitrypsin levels in a range likely to provide antielastase protection for the alveolar structures.

Response of lower respiratory tract α1-antitrypsin levels to the α1-antitrypsin infusions. Because the therapeutic rationale for replacement therapy with α1-antitrypsin is to provide specific protection from the progressive, destructive lung disease associated with this serum antiprotease deficiency, it is important to examine the effect of the intravenous administration of α1-antitrypsin on the extracellular milieu of the alveolar structures. To this end, four of the five participants in this study underwent fiberoptic bronchoscopy and saline lavage before the infusion and again after the second and fourth infusions. While there was no immunochemically detectable α1-antitrypsin in their lower respiratory tract secretions before the administration of the α1-antitrypsin concentrate, all patients had significant levels of α1-antitrypsin per milligram albumin in their lavage fluid 2 d after infusion (Fig. 2, Table II). These lavage α1-antitrypsin values represent

TABLE II

Summary of α1-Antitrypsin Levels in Serum and the Lower Respiratory Tract of the Study Population Treated with α1-Antitrypsin Concentrate

Patient No.	Pretreatment serum α1-antitrypsin	Average serum α1-antitrypsin 2 d after each infusion*	Average serum α1-antitrypsin 7 d after each infusion‡	Average α1-antitrypsin levels in the lower respiratory tract§
		mg/dl		*μg/mg*
1	32	105±10	74±5	29±4
2	42	115±12	79±5	41
3	35	101±15	72±4	—
4	43	104±9	71±6	35±4
5	37	108±12	74±5	36±7

* For each patient, the data represent the mean±SEM of the serum α1-antitrypsin levels on days 2, 9, 16, and 23 of the study protocol; i.e., the serum α1-antitrypsin levels on the second day after each infusion are averaged.

‡ For each patient, the data represents the mean±SEM of the serum α1-antitrypsin levels on days 7, 14, 21, and 28 of the study protocol just before the next infusion; i.e., the serum α1-antitrypsin levels on the seventh day after each infusion are averaged.

§ Bronchoalveolar lavage was performed 2 d after the second and fourth infusions in patients 1, 4, and 5; these values are averaged for each patient; for patient 2, lavage was carried out only once (2 d after the second infusion); lavage was not performed in patient 3. Lower respiratory tract α1-antitrypsin values are presented as micrograms α1-antitrypsin per milligram lavage fluid albumin.

~60% of the α1-antitrypsin per milligram albumin recoverable from PiM subjects (10).

Analysis of the α1-antitrypsin recovered from the alveolar structures during replacement therapy revealed that it displayed the same phenotypic pattern as the α1-antitrypsin plasma fraction administered to the patients, i.e., while the patients were phenotype PiZ, the α1-antitrypsin found on the epithelial surface of their lower respiratory tract 2 d after therapy was of phenotype PiM. The augmented levels of α1-antitrypsin found in the lower respiratory tract after parenteral therapy likely resulted from diffusion of the intravenously administered α1-antitrypsin from the plasma throughout the alveolar structures (data not shown).

Evaluation of the α1-antitrypsin recovered by bronchoalveolar lavage during replacement therapy demonstrated that it was not complexed with protease when studied by crossed immunoelectrophoresis (data not shown). In addition, the functional α1-antitrypsin increased in parallel with the quantity of α1-antitrypsin in the lower respiratory tract during replacement therapy (Figs. 2, 3C, and 3D). Not only does the administered α1-antitrypsin reach the alveolar struc-

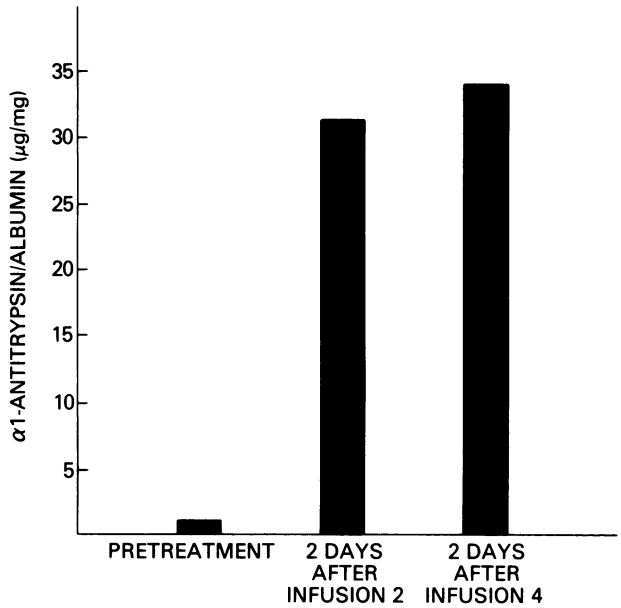

FIGURE 2 Augmentation of lower respiratory tract α1-antitrypsin levels during intravenous replacement therapy with 4.0 g of α1-antitrypsin. In patient 1, lower respiratory tract fluid was recovered by saline lavage before the parenteral administration of α1-antitrypsin (pretreatment) and again 2 d after the second and fourth infusions of 4.0 g of α1-antitrypsin. Lower respiratory tract α1-antitrypsin levels were determined by rocket immunoelectrophoresis on concentrated lavage samples and expressed as a ratio of α1-antitrypsin/lavage fluid albumin (micrograms per milligram) to correct for the variable dilution attendant to the lavage procedure.

tures, but it apparently does so in a form that theoretically can afford an effective antiprotease screen to the lower respiratory tract.

Effects of α1-antitrypsin administration on serum and lower respiratory tract functional antielastase function. Because α1-antitrypsin is the major elastase inhibitor of the lower respiratory tract, the critical clinical effect of α1-antitrypsin deficiency appears to be the loss of elastase inhibitory activity within the alveolar structures. Thus, it is necessary to assess the effect of α1-antitrypsin replacement therapy on the antielastase activity of the lower respiratory tract as a means of confirming the functional activity of the intravenously administered α1-antitrypsin.

The serum antielastase activity of the study population following infusion of α1-antitrypsin concentrate increased in parallel with the α1-antitrypsin antigenic activity (Fig. 3A, B; compare with Fig. 1 and Table II). In a typical patient immediately after infusion, the serum antielastase activity rose from 24% to 82% of normal control serum. At midweek, the serum neutrophil elastase inhibitory activity was 60% of normal; immediately preceding the second infusion it was 35% of normal. On the average, an increment of 18 mg/dl of serum α1-antitrypsin accounted for 10% increase in serum elastase inhibitory activity, similar to that expected in normal individuals. Thus, the infused α1-antitrypsin retained nearly all of its biological activity in vivo.

Determination of the functional activity of the fluid recovered from the alveolar structures of the study population revealed that the lower respiratory tract antielastase activity increased markedly following parenteral α1-antitrypsin replacement. For example, before therapy, lavage fluid from a typical patient inhibited only 15% of the neutrophil elastase activity observed with lavage fluid from a normal, PiM individual. In contrast, following the second and fourth infusions, lavage fluid antielastase activity rose to 60– 70% of normal (Fig. 3C, D). This increase in lower respiratory tract antielastase activity was commensurate with the 60% augmentation of immunochemical α1-antitrypsin, demonstrating that the specific activity of the infused protein is preserved during diffusion into the alveolar structures (Figs. 2, 3C, and 3D). These data confirmed that the infused α1-antitrypsin reached the lower respiratory tract of the PiZ patients in a form that contributes to establishment of elastase-antielastase balance at the level of the alveolar structures.

Hazards of intravenous therapy with the α1-antitrypsin concentrate. In this short-term study during which 320 units of plasma were used to prepare 20 separate infusions of the α1-antitrypsin, there was no evidence of hepatitis transmission. Specifically, there were no abnormalities of liver function tests (serum glutamic oxalacetic transaminase, lactate dehydrogenase, alkaline phosphatase, or bilirubin) for a period of up to 12 mo after the infusion of α1-antitrypsin concentrate. In addition, none of the five patients in the study demonstrated evidence of antibody formation against the PiM protein at periods up to 1 yr following the infusions. The five patients tolerated the osmotic load of the infusions without symptoms or signs of volume overload. No acute allergic reactions were noted following the infusions and no problems were encountered with sterility or pyrogenicity.

DISCUSSION

We have demonstrated a simple method for the preparation of a source of α1-antitrypsin from human plasma suitable for intravenous use in the replacement therapy of severe serum α1-antitrypsin deficiency. Specifically, this approach to α1-antitrypsin deficiency results in reversal of the biochemical defect (i.e., elastase-antielastase imbalance), which likely accounts for the destructive lung disease associated with this serum protein deficiency. Following the infusion of 4.0 g of an α1-antitrypsin concentrate, serum elastase inhibitory activity is maintained for at least 1 wk in a range that putatively affords protection to the lower respiratory

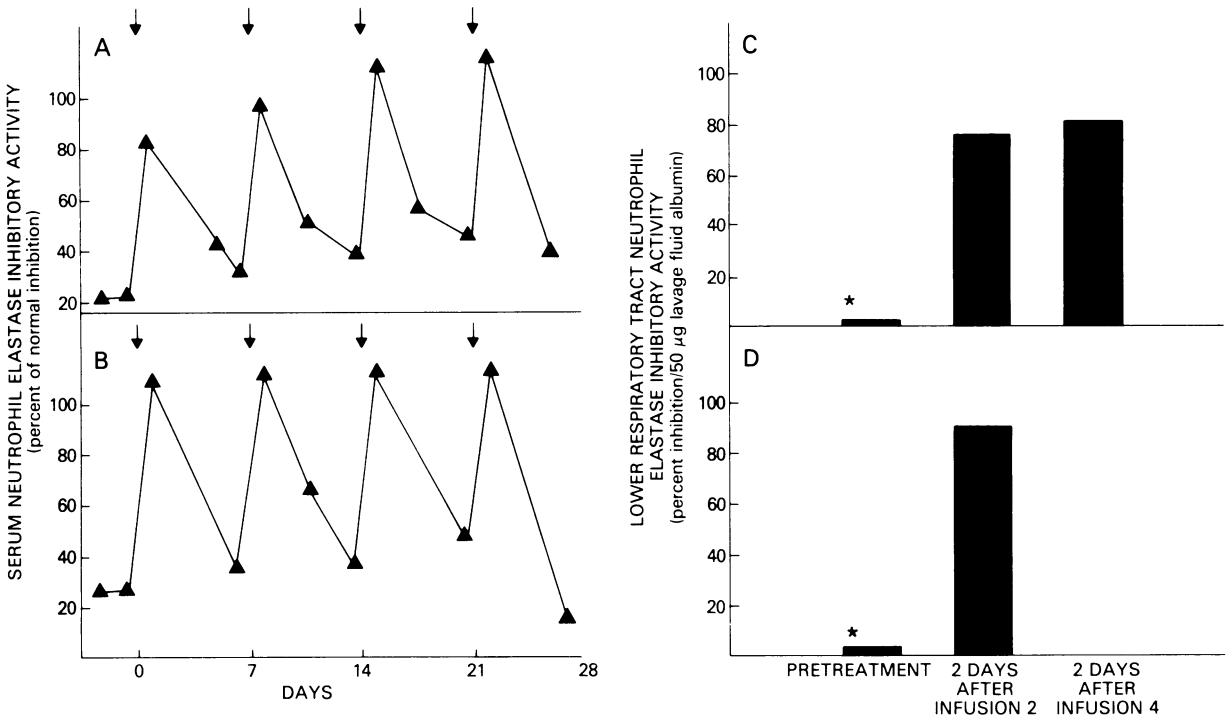

FIGURE 3 Increased serum and lower respiratory tract neutrophil elastase inhibitory activity resulting from parenteral α1-antitrypsin replacement therapy. (A) Serum neutrophil elastase inhibitory activity (expressed as a percent of the inhibitory activity present in normal serum) for patient 1. In this assay, normal human serum inhibits an average of 0.60 μg of elastase per microgram of α1-antitrypsin. (B) Serum neutrophil elastase inhibitory activity for patient 2. Serum inhibitory activity was determined before replacement therapy and at intervals following weekly intravenous infusions of 4.0 g of α1-antitrypsin. Arrows indicate times of α1-antitrypsin infusions relative to the determinations of serum elastase inhibitory activity. (C) Lower respiratory tract neutrophil elastase inhibitory activity of patient 1 expressed as a percent of inhibition of purified elastase per milligram lavage fluid albumin. Elastase inhibitory activity of the lower respiratory tract was determined by evaluating bronchoalveolar lavage fluid before parenteral replacement therapy (pretreatment) and 2 d following the second and fourth infusions. (D) Lower respiratory tract neutrophil elastase inhibitory activity of patient 2 determined before therapy (pretreatment) and 2 d after the second infusion. Asterisk indicates that α1-antitrypsin levels were undetectable immunochemically.

tract from proteolytic attack. In addition, the infusion of this α1-antitrypsin concentrate results in antielastase protection within the alveolar structures.

The clinical significance of this short-term replacement study can be inferred from consideration of the serum and lower respiratory tract α1-antitrypsin activity required to protect against the development of emphysema. While the issue has not been resolved conclusively, it appears that the risk of emphysema associated with the PiMZ phenotype (serum α1-antitrypsin levels 55% of normal) does not greatly exceed that of the normal PiM individual (22–30). In contrast, the protection of the alveolar structures afforded by the serum levels associated with the PiZ phenotype (10–25% of normal) is clearly inadequate since it is estimated that at least 80% of these individuals will develop emphysema (2–4). Among the phenotypes with serum α1-antitrypsin levels midway between the PiZ and PiMZ individuals, the PiSZ phenotype (serum levels 25–35% of normal) has been associated with a form of emphysema indistinguishable from the PiZ disease (31). By comparison, individuals with the PiS phenotype (serum levels 35–50% of normal) do not seem to be at risk for destructive lung disease (3). From these observations, it is possible to infer that the hypothetical "threshold" level of serum α1-antitrypsin necessary to protect against lung proteolysis resides in the range of ~35% of normal. An effective form of replacement therapy should provide α1-antitrypsin levels that are continuously in excess of this threshold value. In this study, the administration of 4 g of α1-antitrypsin intravenously at weekly intervals resulted in the maintenance of serum levels in excess of this level for ~1 wk. By the second infusion, even the "trough" serum α1-antitrypsin levels, just before the third infusion, remained in the range of this postulated

serum α1-antitrypsin level. Concommitant assessment of α1-antitrypsin function and levels recovered from the lower respiratory tract further suggests that a reasonable level of antielastase protection is afforded to the lung with this approach to replacement therapy. In addition, the appearance of functional lung antielastase activity during the course of parenteral α1-antitrypsin replacement further strengthens the concept that α1-antitrypsin is the primary inhibitor of neutrophil elastase within the lower respiratory tract of man (10).

Direct replacement therapy of inherited deficiencies of serum proteins has demonstrated its value in such diverse inherited serum protein deficiencies as hemophilia (32), hypogammaglobulinemia (33), and hereditary angioedema (34). The present study suggests that α1-antitrypsin prepared from pooled, normal human plasma provides a form of this elastase inhibitor with good specific activity and recovery rates that make replacement therapy on a maintenance schedule a feasible undertaking. In addition, the use of polyethylene glycol appears to confer a desirable safety margin with regard to the transmission of hepatitis. Also, there were no significant acute adverse reactions to the infusion or evidence of allotypic sensitization to the infused protein in this short study. By virtue of safety of administration and demonstrated bioactivity, this approach to the therapy of the emphysema associated with severe α1-antitrypsin deficiency appears suitable for testing in long-term studies to determine if reestablishment of elastase-antielastase balance will mitigate the progression of this destructive lung disease (35).

REFERENCES

1. Laurell, C. B., and S. Ericksson. 1963. The electrophoretic α1-globulin pattern of serum in α1-antitrypsin deficiency. *Scand. J. Clin. Lab. Invest.* **15:** 132–140.
2. Eriksson, S. 1964. Pulmonary emphysema and alpha 1-antitrypsin deficiency. *Acta. Med. Scand.* **177:** 175.
3. Kueppers, F., and Black, L. F. α1-Antitrypsin and its deficiency. *Am. Rev. Respir. Dis.* **110:** 176–194.
4. Morse, J. O. 1978. Alpha 1-antitrypsin deficiency. *N. Engl. J. Med.* **299:** 1045–1048, 1099–1105.
5. Janoff, A., B. Sloan, G. Weinbaum, V. Damiano, R. A. Sandhaus, J. Elias, and P. Kimbel. 1977. Experimental emphysema induced with purified human neutro- *Am. Rev. Respir. Dis.* **115:** 461–478.
6. Senior, R. M., H. Tegner, C. Kuhn, et al. 1977. The induction of pulmonary emphysema with human leukocyte elastase. *Am. Rev. Respir. Dis.* **116:** 469–475.
7. Kueppers, F. Inherited differences in alpha 1-antitrypsin. 1978. *In* Genetic Determinants of Pulmonary Disease. S. D. Litwin editor. Marcel Dekker, Inc., New York. 23–74.
8. Jones, E. A., J. Vergalla, C. J. Steer, P. R. Bradley-Moore, and J. M. Vierling. 1978. Metabolism of intact and desalylated α1-antitrypsin. *Clin. Sci. Mol. Med.* **55:** 139–148.
9. Makino, S., and C. E. Reed. 1970. Distribution and elimination of exogenous alpha 1-antitrypsin. *J. Lab. Clin. Med.* **75:** 742–746.
10. Gadek, J. E., G. A. Fells, R. L. Zimmerman, S. I. Rennard, and R. G. Crystal. The antielastases of the human alveolar structures. Implications for the protease-antiprotease theory of emphysema. *J. Clin. Invest.* **68:**
11. Yoshida, A., J. Lieberman, and L. Gaidulis. 1976. Molecular abnormality of human alpha 1-antitrypsin variant (PiZZ) associated with plasma activity deficiency. *Proc. Natl. Acad. Sci. U. S. A.* **73:** 1324–1328.
12. Kueppers, F., and R. J. Fallat. 1969. α1-Antitrypsin deficiency: a defect in protein synthesis. *Clin. Chim. Acta.* **24:** 401–403.
13. Hunninghake, G. W., J. E. Gadek, O. Kawanami, V. J. Ferrans, and R. G. Crystal. 1979. Inflammatory and immune processes in the human lung in health and disease: evaluation by bronchoalveolar lavage. *Am. J. Pathol.* **97:** 149–206.
14. Mancini, G., A. O. Carbonara, and J. F. Heremans. 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry.* **2:** 235–254.
15. Weeke, B. 1973. Rocket immunoelectrophoresis. *In* Quantitative Immunoelectrophoresis. N. Axelsen, J. Kroll, and B. Weeke, editors. Universitetsforlaget, Oslo, Norway. 37–60.
16. Allen, R. C., R. A. Harley, and R. C. Talamo. 1974. A new method for determination of alpha 1-antitrypsin phenotypes using isoelectric focusing on polyacrylamide gel slabs. *Am. J. Clin. Pathol.* **62:** 732–739.
17. Laurell, C. B. 1965. Antigen-antibody crossed electrophoresis. *Anal. Biochem.* **10:** 358.
18. Taylor, J. C., and I. P. Crawford. 1975. Purification and preliminary characterization of human leukocyte elastase. *Arch. Biochem. Biophys.* **169:** 91–101.
19. Gadek, J. E., G. A. Fells, D. G. Wright, and R. G. Crystal. 1980. Human neutrophil elastase functions as a type III collagen "collagenase." *Biochem. Biophys. Res. Commun.* **95:** 1815–1822.
20. Gadek, J. E., G. A. Fells, and R. G. Crystal. 1979. Cigarette smoking induces functional antiprotease deficiency in the lower respiratory tract of humans. *Science (Wash. D. C.).* **206:** 1315–1316.
21. Cawley, L. P., B. Minard, and G. M. Penn. 1978. Counterelectrophoresis. *In* Electrophoresis and Immunochemical Reactions in Gels. Chicago. Amer. Soc. Clin. Pathol. 16–18.
22. Kueppers, F., and A. Donhardt. 1974. Obstructive lung disease in heterozygotes for alpha 1-antitrypsin deficiency. *Ann. Intern. Med.* **80:** 209–212.
23. Klayton, R., R. Fallat, and A. B. Cohen. 1975. Determinants of chronic obstructive pulmonary disease in patients with intermediate levels of α1-antitrypsin. *Am. Rev. Respir. Dis.* **112:** 71–75.
24. Hall. W. J., R. W. Hyde, R. G. Schwartz, G. S. Mudholkar, D. R. Webb, Y. P. Chaubey, and P. L. Townes. 1976. Pulmonary abnormalities in intermediate alpha 1-antitrypsin deficiency. *J. Clin. Invest.* **58:** 1069–1977.
25. Cox, D. W., V. H. Hoeppner, and H. Levison. 1976. Protease inhibitors in patients with chronic obstructive pulmonary disease: the alpha 1-antitrypsin heterozygote controversy. *Am. Rev. Respir. Dis.* **113:** 601–606.
26. Gelb, A. F., E. Klein, and J. Lieberman. 1977. Pulmonary function in nonsmoking subjects with alpha 1-antitrypsin deficiency (MZ phenotype). *Am. J. Med.* **62:** 93–98.

27. Morse, J. O., Liebowitz, M. D., and R. J. Knudson. 1977. Relation of protease inhibitor phenotypes to obstructive lung diseases in a community. *N. Engl. J. Med.* **296:** 1190–1194.
28. Liebowitz, M. D., R. J. Knudson, and J. O. Morse. 1978. Closing volume and flow volume abnormalities in alpha 1-antitrypsin phenotype groups in a community population. *Am. Rev. Respir. Dis.* **117:** 179–181.
29. Mittman, C. 1978. The PiMZ phenotype: is it a risk factor for the development of chronic obstructive lung disease? *Am. Rev. Respir. Dis.* **118:** 649–652.
30. McDonogh, D. J., S. P. Nathan, R. J. Knudson, and D. Lebowitz. 1979. Assessment of alpha 1-antitrypsin deficiency heterozygosity as a risk factor in the etiology of emphysema. *J. Clin. Invest.* **63:** 299–310.
31. Larrson, C., H. Dirksen, G. Sundstrom, et al. 1976. Lung function studies in asymptomatic individuals with moderately (PiSZ) and severely (PiZ) reduced levels of α1-antitrypsin. *Scand. J. Respir. Dis.* **57:** 267–280.
32. Johnson, A. J., M. H. Karpatkin, and J. Newman. 1969. Preparation of and clinical experience with antihemophilic factor concentrates. *Thromb. Diath. Haemorrh. (Suppl.)* **35:** 49–59.
33. Janeway, C. A., and F. S. Rosen. 1966. The gamma globulins. IV. Therapeutic uses of gamma globulin. *N. Engl. J. Med.* **275:** 826–831.
34. Gadek, J. E., S. W. Hosea, J. A. Gelfand, M. Santaella, M. Wickerhauser, D. C. Triantaphyllopoulos, and M. M. Frank. 1980. Replacement therapy in hereditary angioedema. Successful therapy in hereditary angioedema with partially purified Cl inhibitor. *N. Engl. J. Med.* **302:** 542–546.
35. Cohen, A. B. 1979. Opportunities for the development of specific therapeutic agents to treat emphysema. *Am. Rev. Respir. Dis.* **120:** 723–727.