Deficiency of the Chemotactic Factor Inactivator in Human Sera with α_1-Antitrypsin Deficiency

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ABSTRACT As revealed by appropriate fractionation procedures, human serum deficient in α_1-antitrypsin (α_1-AT) is also deficient in the naturally occurring chemotactic factor inactivator. These serum donors had severe pulmonary emphysema. Serum from patients with clinically similar pulmonary disease, but with presence of α_1-AT in the serum, showed no such deficiency of the chemotactic factor inactivator. When normal human serum and α_1-AT-deficient human sera are chemotactically activated by incubation with immune precipitates, substantially more chemotactic activity is generated in α_1-AT-deficient serum. These data indicate that in α_1-AT-deficient serum there is an imbalance in the generation and control of chemotactic factors. It is suggested that the theory regarding development of pulmonary emphysema in patients lacking the α_1-antitrypsin in their serum should be modified to take into account a deficiency of the chemotactic factor inactivator.

INTRODUCTION

The α-globulins of human serum contain several protease inhibitors: α_1-antitrypsin (α_1-AT),[1] α_1-antichymotrypsin, inter-α trypsin inhibitor, and α_2-macroglobulin. The α_1-AT is the major trypsin inhibitor, but can also inhibit bacterial enzymes (1), the neutral protease and elastase (2–4) derived from lysosomal granules of neutrophilic granulocytes, skin collagenase (5), plasmin (6), and thrombin (4). Each of these inhibitors interacts in what appears to be a stoichiometric manner with the target enzyme binding with it and leading to its inactivation. A second type of inhibitor has been described in the α-globulins of human serum: a carboxypeptidase B-like enzyme that inactivates two classes of biologically active peptides: the kinins generated by kallikrein (7) and the anaphylatoxins that emanate as cleavage products from the third and fifth components of complement (8). Because of the ability of the carboxypeptidase B-like enzyme to inactivate the anaphylatoxins, it has been termed the anaphylatoxin inactivator (9). Recently another inactivator has been isolated from the α-globulin region of human serum: the chemotactic factor inactivator (10). This inhibitor appears to act in an enzymatic-like fashion to inactivate complement-derived and complement-independent chemotactic factors.

This paper will record the observation that sera deficient in the α_1 antitrypsin are also deficient in the chemotactic factor inactivator. This finding may bear on the mechanisms responsible for development of pulmonary emphysema in patients who have severe α_1 antitrypsin deficiency.

METHODS

Sera. Five different human sera lacking $>80\%$ of the α_1-AT as judged by measurements of trypsin inhibitory capacity and α_1-AT concentration (11) were used in these studies. By convention, the genotyping of these sera is PiZZ (12). In addition, one serum was used (An) from an individual with no detectable α_1-AT. Each of these patients has severe, chronic pulmonary emphysema and is seriously ill. In order to determine whether the clinical condition of severe pulmonary emphysema is directly related to the status of the chemotactic factor inactivator in serum, sera from six different patients with chronic, progressive pulmonary emphysema were also studied. In each of these sera the level of α_1-AT was at least 50% of the level in normal control serum. Sera from five normal humans, containing $>50\%$ the normal levels of the α_1 antitrypsin, were also used. Some of these preparations were generously provided as fresh frozen sera by Dr. Chester Alper.

Chemotaxis. Modified Boyden chambers employing micropore filters of 650 nm pore size were used for chemotaxis assays (13). The indicator cells were rabbit neutrophilic

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[1] *Abbreviations used in this paper:* α_1-AT, α_1 antitrypsin; BSA, bovine serum albumin.

granulocytes obtained from a 4 h glycogen-induced peritoneal exudate. Cells were suspended in 0.1% bovine serum albumin (BSA) in Hank's medium. Chemotactic factors were diluted in the same medium. Chemotactic values reflect the numbers of migrated neutrophils in a filter, in five high power fields. Details of this technique are given elsewhere (14). In the first part of this study culture filtrates from a 24 h growth of *Escherichia coli* in medium 199 were used as the source of chemotactic factor (15). An amount of 50 μl of this material was mixed with 20 μl Hanks medium or 20 μl chemotactic factor inactivator (see below for details of preparation), incubated for 20 min at 20°C, then diluted to 1.0 ml in Hanks medium for chemotactic assay.

In the other experiments serum (0.1 ml) was chemotactically activated with immune precipitates. The source of the precipitate was 40 μg antibody nitrogen (determined by quantitative precipitin analysis) with antigen (BSA) added at equivalence (8 μg albumin nitrogen). The antibody was isolated as the IgG fraction of serum from hyperimmunized rabbits (16). Chemotactic activation of sera was carried out by incubation of serum with immune precipitates at 37°C for ½ h. Dilutions in Hanks medium were then made for chemotactic testing.

Preparation of chemotactic factor inactivator. Serum was fractionated at room temperature with ammonium sulfate at 45% saturation. The soluble fraction, containing the chemotactic factor inactivator, was dialyzed in phosphate buffered saline, then concentrated with Amicon PM10 membranes (Amicon Corp., Lexington, Mass.) to one-third the original volume of serum. When normal human serum is fractionated, this results in a preparation rich in chemotactic factor inactivator (10). For assay, 20 μl inactivator was added to 50 μl bacterial chemotactic factor and 100 μl phosphate-buffered saline (pH 7.4), the mixture incubated at 25°C for 20 min, then diluted to 1.0 ml in Hanks medium for chemotactic assay.

TABLE I

Inhibition of Bacterial Chemotactic Factor by Human Serum Factor

Serum tested*	Chemotactic activity	Inhibition
		%
α_1-AT-sufficient (normal controls)		
1	45	70
2	20	87
3	40	73
4	45	70
none (reference positive control)	150	—
α_1-AT-deficient (patients with emphysema)		
Bo	130	13
Ba	120	20
An	160	0
Wo	160	0
α_1-AT-sufficient (patients with emphysema)		
Co	5	97
Ne	60	59
Su	45	68
Ti	15	89
Ui	45	72
Ba	40	74

* Conditions of test: soluble fraction of serum after addition of ammonium sulfate at 45% saturation, followed by dialysis against phosphate-buffered saline and concentration of soluble fraction of one third the original volume of serum. 20 μl serum fraction was incubated with 50 μl bacterial factor at 25°C for 20 min, followed by dilution in Hanks medium and chemotactic testing. See text.

RESULTS

Lack of chemotactic factor inactivator in sera-dficient in α_1 antitrypsin. When the soluble ammonium sulfate fractions of four normal human sera were incubated with the bacterial chemotactic factor, 70–80% inhibition of the chemotactic activity resulted (Table I). The loss of activity reflects the action of the chemotactic factor inactivator present in the concentrated fraction of normal serum. The serum fractions from six different patients with chronic, progressive pulmonary emphysema were each found to contain significant inhibitory activity for the chemotactic factor. It was previously determined that in each of these sera there were substantial levels of α_1-AT (see above). In contrast, in spite of the fact that four different human sera, each deficient in the trypsin inhibitor, were fractionated and concentrated in the same manner, inhibitory activity for the bacterial chemotactic factor was lacking, or present in much lower quantity (Table I). The lack of an immunological assay for the chemotactic factor inactivator has not allowed determination of precise levels of the inactivator in the individual sera. These data indicate that serum deficiencies of the chemotactic factor inactivator are associated with α_1-AT deficiency, but not with the clinical condition of pulmonary emphysema per se.

In order to determine if α_1-AT-deficient serum had a blocking effect on the expression of the activity of the chemotactic factor inactivator, 50 μl of the chemotactic factor inactivator (prepared from normal serum according to the details listed above) was added to 50 μl of normal human serum and to an α_1-AT-deficient serum. No loss in the ability of the inactivator to inhibit the chemotactic factor was noted in the two sera (81% inhibition vs. 85% inhibition in the normal and the α_1-AT-deficient serum, respectively). These results suggest that the loss of chemotactic factor inactivator in the α_1-AT-deficient sera is not due to impairment of an existent inactivator in these sera.

The generation of super-normal amounts of chemotactic activity in inhibitor-deficient sera. Since it is now established that the chemotactic factor inactivator inhibits the complement-derived chemotactic factors (C3 and C5 fragments, C$\overline{567}$) as well as the bacterial chemotactic factor (10), it became of interest to determine the

TABLE II

Generation of Chemotactic Activity in Various Human Sera

Serum	Chemotactic activity in serum* 50 μl	25 μl	10 μl
Normal α_1-AT			
1	190	80	20
2	95	60	50
3	205	70	50
4	240	30	40
5	160	120	20
mean‡	178±41	72±22	36±12
Deficient α_1-AT			
6	215	150	130
7	205	160	110
8	190	160	100
9	245	190	100
10	220	130	100
mean	215±14	158±14	108±9

* 0.1 ml serum incubated 30 min at 37°C with BSA-anti-BSA complex, made at antigen equivalence with 40 μg N antibody Volumes represent equivalence of original serum tested.
‡ Mean value ±SEM.

amount of chemotactic activity generated in normal sera and in those sera lacking the chemotactic factor inactivator (along with α_1-AT). This experiment was done in view of the knowledge that when human serum is chemotactically activated, the resulting chemotactic activity is ascribable to C5 products (13, 14), these factors being susceptible to the action of the chemotactic factor inactivator derived from normal human serum (10). The data in Table II compare the amounts of chemotactic activity generated in five normal sera and five α_1-AT-deficient sera after incubation with immune complexes. The amount of chemotactic activity generated in the α_1-AT-deficient sera is two- to threefold greater than the activity generated in normal (α_1-AT-sufficient) human serum. In view of the data in Table I, these findings are not surprising.

DISCUSSION

It can be concluded from these experiments that those sera deficient in the α_1-AT are also deficient in the chemotactic factor inactivator. The most obvious possibility to explain such an association would be identity of the two inhibitors. Evidence so far is against this likelihood. Operationally, the two inhibitors work in quite different ways. The α_1-AT binds to the enzyme in stoichiometric fashion to render it inactive. In studies with the chemotactic factor inactivator, all evidence of binding to the radiotagged C5 chemotactic fragment has been negative (10). It seems probable that the inactivator of chemotactic activity destroys the chemotactic factor in an enzymatic manner, such as kininases destroy kinins (7). A second point that tends to distinguish the two inhibitors is estimates of molecular weight. The α_1-AT has a molecular weight of 45,000, while estimates of the chemotactic factor inactivator suggest the presence of two inhibitors in serum, which may have molecular weights considerably above or below the figure of 45,000 (10). Whether the chemotactic factor inactivator is or is not identical with α_1-AT or with the anaphylatoxin inactivator is a minor consideration in the context of this paper. The message of this report is the lack of the chemotactic factor inactivator in sera deficient in α_1-AT.

The data also suggest that the deficiency of the chemotactic factor inactivator is not associated per se with the condition of pulmonary emphysema, since α_1-AT-sufficient serum from patients with chronic progressive pulmonary emphysema have substantial levels in the serum of the chemotactic factor inactivator. Further, it appears that the lack of chemotactic factor inactivator in α_1-AT-deficient serum is an actual loss of the inactivator rather than its impairment.

It has been suggested that a high percentage of patients with deficiency of α_1-AT develop pulmonary emphysema because the trypsin-like enzymes, including elastase and the neutral proteases derived from lysosomal granules of neutrophilic granulocytes (3), react in an uncontrolled fashion. The data in this paper indicate a second possibility must also be considered: the deficiency or absence of the naturally occurring chemotactic factor inactivator in the serum of the same patients (Table I) could mean an important mechanism for achieving balance of inflammatory responses is missing (Table I). Lacking this control, larger than normal amounts of chemotactic factors would be generated by complement-dependent mechanisms (Table II). With more chemotactic factors being generated and with no natural mechanism to inactive these inflammatory mediators, the stage would be set for inordinate delivery of neutrophils (and their enzymes) to inflammatory exudates. The excessive delivery of cells along with the lack of a natural inhibitor to block the action of trypsin-like enzymes from the leukocytes would make for a highly disadvantageous situation. In view of the data presented here, it would seem appropriate to consider modifying the theories of the pathogenesis of pulmonary emphysema in patients who lack α_1-AT.

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