Deficiency of the Chemotactic Factor Inactivator in Human Sera with \( \alpha_1 \)-Antitrypsin Deficiency

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**Abstract** As revealed by appropriate fractionation procedures, human serum deficient in \( \alpha_1 \)-antitrypsin (\( \alpha_1 \)-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 \( \alpha_1 \)-AT in the serum, showed no such deficiency of the chemotactic factor inactivator. When normal human serum and \( \alpha_1 \)-AT-deficient human sera are chemotactically activated by incubation with immune precipitates, substantially more chemotactic activity is generated in \( \alpha_1 \)-AT-deficient serum. These data indicate that in \( \alpha_1 \)-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 \( \alpha_1 \)-antitrypsin in their serum should be modified to take into account a deficiency of the chemotactic factor inactivator.

**Introduction**

The \( \alpha_1 \)-globulins of human serum contain several protease inhibitors: \( \alpha_1 \)-antitrypsin (\( \alpha_1 \)-AT), \( \alpha_1 \)-antichymotrypsin, inter-\( \alpha \) trypsin inhibitor, and \( \alpha_2 \)-macroglobulin. The \( \alpha_1 \)-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 \( \alpha_1 \)-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 \( \alpha_2 \)-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 \( \alpha_1 \)-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 \( \alpha_1 \)-antitrypsin deficiency.

**Methods**

Sera. Five different human sera lacking \( >80\% \) of the \( \alpha_1 \)-AT as judged by measurements of trypsin inhibitory capacity and \( \alpha_1 \)-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 \( \alpha_1 \)-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 \( \alpha_1 \)-AT was at least 50% of the level in normal control serum. Sera from five normal humans, containing \( >50\% \) the normal levels of the \( \alpha_1 \)-antitrypsin, were also used. Some of these preparations were generously provided as fresh frozen sera by Dr. Chester Alper.

*Chemotaxis.* Modified Boyden chambers employing micro pore filters of 650 nm pore size were used for chemotaxis assays (13). The indicator cells were rabbit neutrophilic.
granulocytes obtained from a 4 h glycogen-induced peri-
toneal 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 re-
fect the numbers of migrated neutrophils in a filter, in five
high power fields. Details of this technique are given else-
where (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 me-
dium 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 chemo-
tactically 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 2 h. Dilutions in Hanks medium were then made
for chemotactic testing.

Preparation of chemotactic factor inactivator. Serum
was fractionated at room temperature with ammonium sul-
fate 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 chemo-
tactic 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 me-
dium for chemotactic assay.

RESULTS

Lack of chemotactic factor inactivator in sera-deficient
in α 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 1). The loss of
activity reflects the action of the chemotactic factor in-
activator 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-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 1). The lack of an immunological assay for the
chemotactic factor inactivator has not allowed deter-
mination of precise levels of the inactivator in the indi-
vidual sera. These data indicate that serum deficiencies
of the chemotactic factor inactivator are associated with
α-AT deficiency, but not with the clinical condition of
pulmonary emphysema per se.

In order to determine if α-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 accord-
ing to the details listed above) was added to 50 μl of
normal human serum and to an α-AT-deficient serum.
No loss in the ability of the inactivator to inhibit the
chemotactic factor was noted in the two sera (81% inhib-
ition vs. 85% inhibition in the normal and the α-AT-
deficient serum, respectively). These results suggest that
the loss of chemotactic factor inactivator in the α-AT-
deficient sera is not due to impairment of an existent
inactivator in these sera.

The generation of super-normal amounts of chemo-
tactic activity in inhibitor-deficient sera. Since it is now
established that the chemotactic factor inactivator in-
hibits the complement-derived chemotactic factors (C3 and
C5 fragments, C567) as well as the bacterial chemo-
tactic factor (10), it became of interest to determine the

| Table 1 |

<table>
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<th>Serum tested*</th>
<th>Chemotactic activity</th>
<th>Inhibition</th>
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<td>a1-AT-sufficient (normal controls)</td>
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<td>4</td>
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* 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.
amount of chemotactic activity generated in normal sera and in those sera lacking the chemotactic factor inactivator (along with α1-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 α1-AT-deficient sera after incubation with immune complexes. The amount of chemotactic activity generated in the α1-AT-deficient sera is two- to threefold greater than the activity generated in normal (α1-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 α1-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 α1-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 kininas destroy kinins (7). A second point that tends to distinguish the two inhibitors is estimates of molecular weight. The α1-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 α1-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 α1-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 α1-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 α1-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 α1-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 inactivate 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 α1-AT.

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REFERENCES


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