THE EFFECT OF $\alpha$1 ANTITRYPSIN ON PHAGOCYTE FUNCTION

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SUMMARY $\alpha$1 antitrypsin ($\alpha$1 AT) deficiency is a common genetic variant associated with many immunological and inflammatory disorders. Previous studies in $\alpha$1 AT deficient subjects have indicated possible monocyte (MN) and neutrophil (PMN) abnormalities with enhanced chemiluminescence (CL) and accelerated delayed hypersensitivity reactions. We have therefore extended these observations by investigating the effect of purified $\alpha$1 AT on MN and PMN motility and CL. These studies indicated that $\alpha$1 AT in physiological amounts inhibits PMN and MN chemokinetics by about 35%, but chemotaxis by only 10%. It however has no direct effect on the zymosan induced CL of these cells. This study suggests that the increased incidence and severity of some inflammatory diseases in $\alpha$1 AT deficient subjects may in part be explained by increased motility and activation of MN and PMN, the former due to the absence of an inhibitor of PMN and MN locomotion and the latter due to secondary changes in serum resulting in enhanced chemiluminescence.

INTRODUCTION

$\alpha$1 ANTITRYPSIN ($\alpha$1 AT) deficiency is a common genetic variant being found in about 10% of most caucasian populations. Such people are subject to a number of inflammatory diseases including rheumatoid arthritis (RA) (1, 2, 3), systemic lupus erythematosus (SLE) (1), juvenile chronic polyarthritis (JCP) (4), anterior uveitis (AU) (5, 6), fibrosing alveolitis (7) and asthma (8). Asthma, AU and possibly RA also appears to be more severe in $\alpha$1 AT deficient subjects compared with their non deficient counterparts.

As part of a study designed to investigate the immune and inflammatory abnormalities predisposing to these diseases, we have previously demonstrated, in $\alpha$1 AT deficient subjects, accelerated delayed hypersensitivity responses (DTH) and enhanced zymosan induced chemiluminescence (CL) responses of mononuclear (MN) cells and neutrophils (PMN) in the presence of their serum (9). We have now investigated MN and PMN function in more detail by utilising purified $\alpha$1 AT to reseass its action on CL and to investigate its effect on chemotaxis, another important aspect of phagocyte function. Our results indicate that purified $\alpha$1 AT inhibits predominantly the chemokinetic response of both PMN and MN but has no direct effect on zymosan induced CL of these cells. This would suggest that our earlier findings of enhanced CL in the presence of $\alpha$1 AT deficient serum may be due to changes in other serum proteins, such as complement, rather than $\alpha$1 AT itself.

MATERIALS AND METHODS

Purification of $\alpha$1 AT

This was carried out essentially as described by Laurell (10) utilising the thiol disulphide interchange reaction between $\alpha$1 AT and 3-carboxy-4-nitrobenzenesulphonic modified glutathione sepharose. Extensive immunabsorption with specific antisera removes small quantities of remaining impurities, predominantly albumin and prealbumin. $\alpha$1 AT thus purified exhibits normal antitryptic activity utilizing the method of Dietz et al. (11). Immunoelectrophoresis of a highly concentrated sample with an antibody to whole serum reveals only a single arc, due to $\alpha$1 AT.

Cell Separation. isolation of MN and PMN from control subjects was carried out by the standard techniques of Ficoll Hypaque density gradient centrifugation and dextran sedimentation. Cells were washed 3 times in Hanks Balanced Salt Solution (HBSS) (Flow Laboratories N.S.W., Australia) prior to use.

Serum

This was obtained from subjects with asymptomatic $\alpha$1 AT deficiency (genotype $\alpha$1Z and level 0.2-0.3 g/l) and also normal controls (2-4 g/l).

Chemiluminescence

This was carried out for both MN and PMN as previously described (12). In brief, $2 \times 10^6$ MN or PMN in 0.2 ml of HBSS were placed in vials in the presence of 0.05 ml of 0.1 M, 1% hemolysis 0.4 M 1 ml of phosphate buffered saline (PBS) and 0.144 ml of normal or $\alpha$1 AT deficient serum. To the PBS was added sufficient purified $\alpha$1 AT to raise the level within the serum supplement by either 0, 0.8, 1.3, 3.3 or 4.8 g/l. These vials were
counted repeatedly in a liquid scintillation counter to obtain a baseline value. 0.2 ml of zymosan (15 mg/ml) was then added to activate the cells which were then counted for 6 sec every 2 min for 1 hr. The peak response was then obtained for each sample.

MN and PMN Motility

This was carried out using the standard Boyden chamber system as described elsewhere (13). A cell concentration of 2 x 10⁶/ml was used for PMN and 2.5 x 10⁵/ml for MN. Casein 2.5 mg/ml in HBSS was used as a chemoattractant and 5 mg/ml z1 AT deficient serum added to top and bottom chambers for assessment of chemokinesis. With the exception of the chemokinetic assays, all assays were undertaken without serum. To assess the effect of z1 AT it was incubated with cells for 30 min at 37°C at a physiological concentration of 3 g/l. Migration was then allowed to occur at 37°C for 30 min for PMN and 150 min for MN through 3.5 micron and 8 micron millipore filters (Millipore Corporation Bedford, Ma.) respectively.

The filters were then removed, stained, mounted and migration of the leading front measured in microns. Five migration distances were measured at separate locations on each filter. Three sets of experiments were carried out and all manipulations were undertaken in triplicate.

Results

Chemiluminescence

We have previously demonstrated that the CL response of MN and PMN was enhanced in the presence of z1 AT deficient serum. To determine if this effect was due to z1 AT we used purified protein to raise the z1 AT in the serum of deficient subjects from 0.2 g/l to 5 g/l and in normal serum from 2.5 to 7 g/l. In three separate experiments, one of which is seen in Figure 1, there was no suppression of the chemiluminescence response indicating that z1 AT itself was not responsible for the enhanced CL. This suggests that other factors in the serum secondary to z1 AT deficiency were in fact responsible for this finding.

Cell Motility

As can be seen from Table 1, with addition of purified z1 AT there is a small decrease in chemotaxis of both PMN (mean of 74 versus 68 microns) and MN (mean of 96 versus 86 microns) but a much larger decrease of about 35% in their chemokinesis. (PMN 64 versus 41 microns and MN 65 versus 44 microns.) These were significant using 2 way analysis of variance with p < 0.001. There was no noticeable effect of z1 AT on the random migration of these cells. These studies indicate that z1 AT in physiological doses inhibits the chemokinetic response of MN and PMN induced by 5% serum. In all likelihood casein not only stimulates chemotaxis but also chemokinesis. Therefore, the very small (10%) but significant inhibition of casein induced chemotaxis of these cells by z1 AT, in fact probably also represents inhibition of chemokinesis.

Discussion

Phagocytic cells such as MN and PMN are usually the direct mediators of tissue injury in inflammatory lesions irrespective of the initiating event. Anything influencing the motility and activation of these cells is likely to be able to influence the resultant inflammatory response. There is considerable evidence that cell surface serine esterases are involved in the activation of these phagocytic cells and some of this evidence has been reviewed (9). Our CL results do not support the hypothesis that z1 AT acts on any of these enzymes. This suggests that our earlier findings of enhanced CL in z1 AT deficient subjects is due to other secondary factors. We have previously reported these subjects had elevated levels of the important complement proteins C3, C5 and factor B (9). Whilst the number of patients were small, in general, patients with the highest levels of these proteins tended to have relatively greater CL responses. As this is a complement dependent assay, this suggests the elevation of these proteins may have been responsible for the increased CL in this group.

The role of proteases and protease inhibitors in phagocytic cell motility was first appreciated by Ward and Becker when they demonstrated that 2 esterases, inhibitable by DFP, were involved in the chemotaxis of human PMN (14). One esterase was present in an active form and the other required activation. Niedel has shown that synthetic inhibitor TPCK but not TLCK inhibited PMN chemotaxis to formyl methionyl peptides, suggesting the role of a chymotrypsin-like enzyme in this process (15). Several other workers (16, 17, 18) have demonstrated
synthetic protease inhibitors interfere with the activation and aggregation of phagocytic cells induced by chemotactic substances. Additionally serine esterases may play a part in the inactivation of various chemotactants (19).

Only one previous study has investigated the role of any mammalian plasma proteases inhibitors in regulating PMN motility. In 1975 Groetzl (20) reported a fatal complex effect of α1AT on C3a and C5a induced PMN chemotaxis. He found that cells pulsed with α1AT for a very short duration exhibited enhanced random migration and decreased chemotaxis. More prolonged incubation resulted in abrogation of this effect. Similar effects were found with α1 macroglobulin TPCk and TLCK but no C1 inhibitor. However, the purity and functional activity of #1 AT was not specified, and this is important considering the difficulties involved in its isolation. The dose of α1AT (0.1 g/l) used by him is very small considering its normal serum level of 2–4 g/l.

The work of Keller and colleagues (21) strongly suggests that two different mechanisms regulate the migration of PMN. Indeed they suggest that chemokinetic factors determine the speed of locomotion of the cell and chemotactic factors only give the movement a specified direction. In vitro pure chemotactic factors cause no cell migration alone and can only be effective in the presence of a chemokinetic signal. In vivo this distinction is irrelevant since serum contains ample chemokinetic factors. Our work therefore suggests that α1AT has a very significant effect on the locomotory function of phagocytic cells through its influence on chemokinetics. Presumably α1AT inhibits a cell membrane serine esterase which is activated by a chemokinetic signal. Our earlier findings of accelerated DTH in α1AT deficiency could therefore conceivably represent an in vitro corollary of this reaction, representing accelerated locomotion of macrophages.

This study suggests that the increased incidence and severity of some inflammatory diseases in α1AT deficient subjects may in part be explained by increased motility and activation of MN and PMN, the former due to the absence of an inhibitor of PMN and MN locomotion and the latter due to secondary changes in serum resulting in enhanced chemiluminescence.

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REFERENCES


