THE EFFECT OF α1 ANTITRYPSIN ON PHAGOCYTE FUNCTION

S. N. BREIT, J. P. ROBINSON and R. PENNY

Department of Immunology, St. Vincent's Hospital, and Department of Medicine, University of New South Wales, Sydney, Australia

SUMMARY α1 antitrypsin (α1AT) deficiency is a common genetic variant associated with many immunological and inflammatory disorders. Previous studies in α1 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 α1 AT on MN and PMN motility and CL.. These studies indicated that α1 AT in physiological amounts inhibits PMN and MN chemokinesis 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 α1 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

α1 ANTITRYPSIN (α1 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 α1 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 α1 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 α1 AT to reassess its action on CL and to investigate its effect on chemotaxis, another important aspect of phagocyte function. Our results indicate that purified α1 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

Correspondence: S. N. Breit, Department of Immunology. St. Vincent's Hospital. Sydney, 2010, Australia.

the presence of α1 AT deficient serum may be due to changes in other serum proteins, such as complement, rather than α1 AT itself.

MATERIALS AND METHODS

Purification of α1 AT

This was carried out essentially as described by Laurell (10) utilising the thiol disulphide interchange reaction between α1 AT and 3-carboxy-4-nitrobenzenthiol modified glutathione sepharose. Extensive immuno-absorption with specific antisera removes small quantities of remaining impurities, predominantly albumin and prealbumin. α1 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 α1 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 subject with asymptomatic α1 AT deficiency (phenotype PiZZ 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, 2×10^5 MN or PMN in 0·2 ml of HBSS were placed in vials in the presence of 0·05 ml of 0·1% luminol 0·41 mls of phosphate buffered saline (PBS) and 0·14 ml of normal or α1 AT deficient serum. To the PBS was added sufficient purified α1 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 (5 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 2×10^6/ml was used for PMN and $2·5 \times 10^6$/ml for MN. Casein 2·5 mg/ml in HBSS was used as a chemotractant and 5% α1 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 α1 AT it was incubated with cells for 30 min at 37°C at a physiological concentration of 3g/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

Chemiluminesence

We have previously demonstrated that the CL response of MN and PMN was enhanced in the presence of α1 AT deficient serum. To determine if this effect was due to α1 AT we used purified protein to raise the α1 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 α1 AT itself was not responsible for the enhanced CL. This suggests that other factors in the serum secondary to α1 AT deficiency were in fact responsible for this finding.

Cell Motility

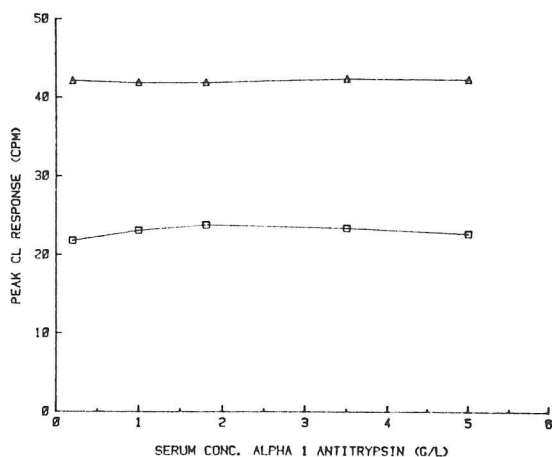As can be seen from Table 1, with addition of purified α1 AT there is a small decrease in chemotaxis of both

FIG. 1. Addition of increasing amounts of purified α1 AT to α1 AT deficient serum causes no suppression of the peak CL response of PMN (△) or MN (□).

Table 1   Effect of α1 AT on monocyte and neutrophil motility

		Mean distance without α1 AT	Mean distance with α1 AT
Chemokinesis	MN	65	44
	PMN	64	41
Chemotaxis	MN	96	86
	PMN	74	68

PMN (mean of 74 versus 68 microns) and MN (mean of 96 v. 86 microns) but a much larger decrease (of about 35%) in their chemokinesis. (PMN 64 v. 41 microns and MN 65 v. 44 microns.) These were significant using 2 way analysis of variance with $p < 0·001$. There was no noticeable effect of α1 AT on the random migration of these cells. These studies indicate that α1 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 α1 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 α1 AT acts on any of these enzymes. This suggests that our earlier findings of enhanced CL in α1 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 methiony 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 chemotractants (19).

Only one previous study has investigated the role of any mammalian plasma proteases inhibitors in regulating PMN motility. In 1975 Goetzl (20) reported a rather complex effect of $\alpha1$ AT on C3a and C5a induced PMN chemotaxis. He found that cells pulsed with $\alpha1$ AT 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 $\alpha2$ macroglobulin TPCK and TLCK but no C1 inhibitor. However, the purity and functional activity of his $\alpha1$ AT was not specified, and this is important considering the difficulties involved in its isolation. The dose of $\alpha1$ AT (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 $\alpha1$ AT has a very significant effect on the locomotory function of phagocytic cells through its influence on chemokinesis. Presumably $\alpha1$ AT inhibits a cell membrane serine esterase which is activated by a chemokinetic signal. Our earlier findings of accelerated DTH in $\alpha1$ AT deficiency could therefore conceivably represent an *in vivo* corollary of this reaction, representing accelerated locomotion of macrophages.

This study suggests that the increased incidence and severity of some inflammatory diseases in $\alpha1$ 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.

ACKNOWLEDGEMENTS

REFERENCES

1. Breit, S. N., Clark, P. and Penny, R. (1980). $\alpha1$ Protease inhibitor ($\alpha1$ antitrypsin) phenotypes in rheumatic disease. *Aust. N.Z. J. Med.*, **10**, 271.

2. Cox, D. W. and Huber, O. (1976). Rheumatoid arthritis and alpha 1 antitrypsin *Lancet*, **i**, 1216.

3. Buisseret, P. D., Pembrey, M. E. and Lessor, M. H. (1977). $\alpha1$ antitrypsin phenotypes in rheumatoid arthritis and ankylosing spondylitis. *Lancet*, **ii**, 1358.

4. Arnaud, P., Galbraith, R. M., Faulk, W. P. and Ansell, B. M. (1977). Increased frequency of the MZ phenotype of alpha 1, protease inhibitor in juvenile chronic polyarthritis. *J. Clin. Invest.*, **60**, 1422.

5. Wakefield, D., Breit, S. N., Clark, P. and Penny, R. Immunogenetic factors in inflammatory eye disease. *Arthritis and Rheum.* (in press).

6. Brewerton, D. A., Webley, M., Murphy, A. H. and Milford-Ward, A. (1978). The $\alpha1$ antitrypsin phenotype MZ in acute anterior uveitis. *Lancet*, **i**, 1103.

7. Geddes, D. M., Webley, M., Brewerton, D. A., Turton, C. W., Turner-Warwick, M., Murphy, A. H. and Milford-Ward, A. (1977). $\alpha1$ antitrypsin phenotypes in fibrosing alveolitis and rheumatoid arthritis. *Lancet*, **ii**, 1049.

8. Arnaud, P., Chapuis-Cellier, C., Souilett, G., Carron, R., Wilson, G. B., Creyssel, R. and Fudenberg, H. H. (1976). High frequency of deficient Pi phenotypes of alpha 1 antitrypsin in nonatopic infantile asthma *Trans. Ass. Amer. Phycns.*, **89**, 205.

9. Breit, S. N., Robinson, J. P., Luckhurst, E., Clark, P. and Penny, R. (1982). Immunoregulation by $\alpha1$ antitrypsin. *J. Clin. Lab. Immunol.*, **7**, 127.

10. Laurell, C. B., Pierce, J., Persson, U. and Thulin, E. (1975). Purification of $\alpha1$ antitrypsin from plasma through that thiol-disulfied interchange *Eur. J. Biochem.*, **57**, 107.

11. Dietz, A. A., Rubinstein, H. M. and Hodges, L. (1974). Measurement of $\alpha1$ antitrypsin in serum by immunodiffusion and by enzymatic assay. *Clin. Chem.*, **20**, 396.

12. Robinson, J. P. and Penny, R. (1982). Chemiluminescence response in normal human phagocytes. Automated measurements using a standard liquid scintillation counter. *J. Clin. Lab. Immunol.*, **7**, 215.

13. McIntosh, J., Hansen, P., Ziegler, J. B. and Penny, R. (1976). Defective immune and phagocyte functions in uraemia and renal transplantation. *Int. Archs. Allergy Appl. Immunol.*, **51**, 544.

14. Ward, P. A. and Becker, E. L. (1967) Mechanisms of the inhibition of chemotaxis by phosphonate esters. *J. Exp. Med.*, **125**, 1001.

15. Niedel, J., Frothingham, R. and Cuatrecasas, R. (1980). Inhibition of ^{125}I-chemotactic peptide uptake by protease inhibitors. *Biochem. Biophys. Res. Commun.*, **94**, 667.

16. Kitagawa, S., Takaku, F. and Sakamoto, S. (1979). Serine protease inhibitors inhibit superoxide production by human polymorphonuclear leukocytes and monocytes stimulated by various surface active agents. *FEBS Letters*, **107**, 331.

17. Damerau, B., Grunefeld, E. and Vogt, W. (1980). Aggregation of leukocytes induced by the complement derived peptides C3a and C5a and by three synthetic formyl-methionyl peptides. *Int. Archs. Allergy Appl. Immun.*, **63**, 159.

18. Simchowitz, L., Mehta, J. and Spilberg, I. (1979). Chemotactic factor-induced superoxide radical generation by human neutrophils. Requirement for proteinase (esterase) activity. *J. Lab. Clin. Med.*, **94**, 403.

19. Brozna, J. P., Senior, R. M., Kreutzer, D. L. and Ward, P. A. (1977). Chemotactic factor inactivators of human granulocytes. *J. Clin. Invest.*, **60**, 1280.

20. Goetzl, E. J. (1975). Modulation of human neutrophil polymorphonuclear leukocyte migration by human plasma globulin inhibitors and synthetic esterase inhibitors. *Immunology*, **29**, 163.

21. Killer, H. V., Wissler, J. H., Hess, M. W. and Cottier, H. (1978). Distinct chemokinetic and chemotactic responses in neutrophil granulocytes. *Eur. J. Immunol.*, **8**, 1.