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Modulation of Human Neutrophil Polymorphonuclear Leucocyte Migration by Human Plasma Alpha-Globulin Inhibitors and Synthetic Esterase Inhibitors

E. J. GOETZL

Departments of Medicine, Harvard Medical School, Robert B. Brigham Hospital, Boston, Massachusetts, U.S.A.

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Summary. The exposure of isolated washed human neutrophils to purified human α1-antitrypsin resulted in a transient 2-fold enhancement of random migration and concomitant 70–90 per cent inhibition of chemotactic responsiveness to C5a or C3a, while treatment with α2-macroglobulin gave a less pronounced brief enhancement of random migration and prolonged 40–60 per cent suppression of chemotaxis. Peak effects occurred with concentrations of 1 μg/ml of α1-antitrypsin and 10 μg/ml of α2-macroglobulin. In contrast, the inhibitor of the activated first component of complement, at the highest concentration studied of 100 μg/ml, slightly enhanced chemotactic migration in response to C5a without influencing random migration. Preincubation of neutrophils with either L-1-tosylamide-2-phenylethyl-chloromethyl ketone (TPCK) or *N*-α-*p*-tosyl-L-lysine-chloromethyl ketone (TLCK) at concentrations of 10^{-8}–10^{-4}M suppressed chemotaxis with concomitant inhibition of random migration by TPCK and enhancement of random migration by TLCK. All agents worked directly and irreversibly on the cells but caused only slight stimulation of the activity of the hexose monophosphate shunt of layers of adherent neutrophils. The results suggest that interaction of the plasma α-globulins or synthetic esterase inhibitors with surface receptors on neutrophils can influence both the random migration and responsiveness to chemotactic factors of these cells.

INTRODUCTION

Plasma α-globulins can regulate some critical fluid-phase enzymatic reactions either by binding to the enzymes to form complexes which exhibit both reduced enzyme and inhibitor activities (Harpel, 1973; Schreiber, Kaplan, and Austen, 1973a), or by inactivating the enzymes without loss of inhibitor functional integrity (Schreiber, Kaplan and Austen, 1973b). C1INH, the α-globulin inhibitor of the activated first component of complement, also inhibits kallikrein, plasmin, and plasma thromboplastin antecedent (PTA), and blocks the ability of Hageman factor fragments to activate prekallikrein, plasminogen proactivator, and pre-plasma thromboplastin antecedent (pre-PTA) (Gigli, Ruddy and Austen,

Correspondence: Dr E. J. Goetzl, Departments of Medicine, Harvard Medical School, Robert B. Brigham Hospital, 125 Parker Hill Avenue, Boston, Massachusetts 02120, U.S.A.

1968; Ratnoff, Pensky, Ogston, and Naff, 1969; Forbes, Pensky and Ratnoff, 1970; Schreiber *et al.*, 1973b). Alpha1-antitrypsin (α1-AT) and alpha2-macroglobulin (α2-M) both demonstrate broad inhibitory specificity for plasma enzymes (Gans and Tan, 1968; Schreiber *et al.*, 1973a; Heck and Kaplan, 1974), as well as a capacity to inhibit enzymes present in human neutrophils including spontaneous fibrinolysin, neutral protease and elastase (Janoff, 1972). α1-AT also suppresses the activity of a human neutrophil membrane-associated protease which can generate a neutral peptide mediator of inflammation by cleavage of a plasma protein substrate (Wintroub, Goetzl and Austen, 1974). The interaction of chemotactic factors with rabbit polymorphonuclear leucocytes results in the activation of a leucocyte chymotrypsin-like esterase which is required for chemotactic migration based on the parallel inhibition by phosphonates of chemotaxis and esterase activity (Ward and Becker, 1968, 1970). In view of the essential role of esterases in neutrophil motility (Ward and Becker, 1968, 1970), the present study was carried out to assess the influence of purified human α-globulin inhibitors, C1INH, α1-AT, α2-M and the synthetic esterase inhibitors, *N*-α-*p*-tosyl-L-lysine-chloromethyl ketone (TLCK) and L-1-tosylamide-2-phenylethylchloromethyl ketone (TPCK), on the random and chemotactic migration of isolated human neutrophils.

MATERIALS AND METHODS

Acrylic radioassay migration chambers (Neuro Probe, Incorporated, Bethesda, Maryland) and disposable polystyrene modified Boyden chambers (Adaps, Incorporated, Dedham, Massachusetts) were assembled with micropore filters (Millipore Corporation, Bedford, Massachusetts) as previously described (Goetzl and Austen, 1972a, b). Hanks's solution and Medium 199 with or without phenol red (Microbiological Associates, Bethesda, Maryland), ovalbumin five times recrystallized (Miles-Seravac, Kankakee, Illinois), dextran, Sephadex, sulphopropyl (SP) and quaternary aminoethyl (QAE) Sephadex, Sepharose and Ficoll (Pharmacia Fine Chemicals, Incorporated, Piscataway, New Jersey), sodium diatrizoate (Hypaque, Winthrop Laboratories, New York), purified Jack Bean concanavalin A, chromatographically purified soybean trypsin inhibitor, lima bean trypsin inhibitor, twice recrystallized trypsin previously treated with diphenyl carbamyl chloride, L-1-tosylamide-2-phenylethyl-chloromethyl ketone (TPCK), and *N*-α-*p*-tosyl-L-lysine-chloromethyl ketone (TLCK) (Sigma Chemical Company, St Louis, Missouri), hexadimethrine bromide (Aldrich Chemical Company, Incorporated, Milwaukee, Wisconsin), sodium [^{51}Cr]chromate and [1-^{14}C]glucose (Amersham-Searle Corporation, Arlington Heights, Illinois), sodium lauryl sulphate (Fisher Scientific Company, Bedford, Massachusetts), plastic 35 × 10 mm Petri dishes (Falcon Plastics, Oxnard, California), cyanogen bromide (Eastman Kodak Company, Rochester, New York), antisera to α1-antitrypsin and α2-macroglobulin (Behring Diagnostics, Incorporated, Woodbury, New York), and methyl-α-D-glucopyranoside (Pfanstiehl Laboratories, Incorporated, Waukegan, Illinois), were obtained as noted. TPCK was recrystallized twice from 95 per cent ethanol. Radial immunodiffusion plates containing anti-human C1INH were a gift from Dr Peter H. Schur (Schur, Connelly and Jones, 1975).

Preparation of α-globulins

C1INH was purified from 100 ml of plasma separated from blood collected in ethylenediamine tetra-acetate (EDTA) and hexadimethrine as previously described (Schreiber

et al., 1973b). After dialysis against 0·0035 M sodium phosphate buffer, pH 7·8, the plasma was applied to a QAE–Sephadex column equilibrated with the same buffer and developed with a linear NaCl gradient. C1INH eluted in a single peak, at a NaCl concentration of 0·15–0·17 M, as assessed by immunodiffusion or function (Gigli *et al.*, 1968) in microtitre plates. This material was pooled, concentrated and further purified by sequential chromatography on SP–Sephadex, Sephadex G–200, and Sephadex G-150; alkaline disc gel electrophoresis yielded one predominant and one lesser band which corresponded to the C1INH activity as previously reported (Schreiber *et al.*, 1973b). Another preparation of highly purified human C1INH obtained as a gift from Dr David H. Bing had been prepared by sequential chromatography followed by elution from a Sepharose–anti-human C1INH affinity column; this material had only 20–30 per cent of the activity (Gigli *et al.*, 1968) of an equivalent weight of C1INH prepared by the former method.

α1-AT and α2-M were purified from pooled fractions of the same QAE–Sephadex column chromatography of human plasma as was utilized to isolate C1INH; counter-electroimmunodiffusion was employed to assay semiquantitatively the presence of each protein in column fractions (Gocke and Howe, 1970). The ascending half of the peak of α1-AT eluting at 0·09–0·12 M NaCl was further purified either by sequential chromatography on SP–Sephadex and Sephadex G-100 as described (Schreiber *et al.*, 1973a) or by affinity chromatography on concanavalin A–Sepharose with elution of α1-AT by 0·1 M methyl-α-D-glucopyranoside (Liener, Garrison and Pravda, 1973), followed by Sephadex G-100 gel filtration. Alkaline disc gel electrophoresis showed a heavy albumin band only in the former preparation, but neither C1INH nor α2-M was detected by radial immunodiffusion in either purified α1-AT preparation. The QAE–Sephadex peak of α2-M eluting at 0·05–0·09 M NaCl was further purified by sequential chromatography on Sephadex G-200 and Sepharose 6-B in the opposite order to that previously described (Schreiber *et al.*, 1973a). This material showed one predominant band of α2-M on disc gel electrophoresis and contained neither α1-AT nor C1INH by radial immunodiffusion. The concentrations of C1INH, α1-AT and α2-M in the purified preparations used were determined by radial immunodiffusion (Mancini, Carbonara and Heremans, 1965).

Neutrophil migration

Human peripheral leucocytes obtained from normal donors by previously detailed methods (Goetzl and Austen, 1972a) were suspended in Medium 199, layered on Ficoll–Hypaque cushions, and centrifuged to separate the mononuclear cells from the neutrophils (Böyum, 1968). Purified neutrophil pellets containing 95–98 per cent neutrophils were then washed twice with Medium 199 made 0·4 g/100 ml in ovalbumin (Medium 199–ovalbumin) and resuspended in the same medium at a concentration of $5{\cdot}0 \pm 0{\cdot}5 \times 10^6$ cells/ml for the modified Boyden assay (Goetzl and Austen, 1972a). Neutrophils utilized for the radiomigration assay were labelled with [^{51}Cr]sodium chromate (Goetzl and Austen, 1972b), washed three times and resuspended in Medium 199–ovalbumin at a concentration of $6{\cdot}0 \pm 0{\cdot}5 \times 10^6$ cells/0·5 ml with 60–80,000 counts per 4 minutes per 0·5 ml. The chemotactic factors utilized were C5a and C3a made by tryptic digestion (Ward and Newman, 1969) of highly purified C5 and C3 (Nilsson and Müller-Eberhard, 1965), respectively. The digestion mixtures were heated for 30 minutes at 61° to inactivate the trypsin and were then centrifuged to separate precipitated protein from the supernatant solution of C5a and C3a.

Chemotactic and random migration assays were modified to enable an investigation of

the initial interaction between neutrophils and α-globulins or synthetic esterase inhibitors by employing high cell concentrations, 8-μm pore filters for the modified Boyden assay or a layer of two 8-μm pore filters for the radiomigration assay, and short incubation times. Boyden chambers were incubated for 50–60 minutes in chemotactic assays and for 80–90 minutes in random migration assays without a chemotactic stimulus. Radiomigration chambers were incubated for 90–120 minutes for chemotaxis and for 150–180 minutes for random migration. Modified Boyden chemotaxis and random migration assessed in duplicate chambers were quantified as the mean number of neutrophils per high power field (hpf) which had migrated 80 μm into the filters from the cell source at the top, minus unstimulated background counts for the chemotactic assay. Radioactive assays of chemotaxis and random migration were expressed as the radioactivity (counts/4 minutes) in the bottom filter away from the cell source per 50,000 counts/4 minutes in the initial cell suspension, with a background subtraction for the chemotactic assay. The distribution of cells within micropore filters was determined by microscopic counts at various depths as described by Zigmond and Hirsch (1973).

Hexose monophosphate shunt (HMPS) activity of adherent neutrophils

The activity of the HMPS of layers of purified neutrophils adherent to plastic Petri dishes was determined by measuring the rate of conversion of [1-^{14}C]glucose to $^{14}CO_2$ in 1 hour under conditions where no [6-^{14}C]glucose was converted to $^{14}CO_2$ (Goetzl and Austen, 1974). The mean ct/minute of $^{14}CO_2$ generated in duplicate dishes were divided by the mean optical density at 280 nm of the 3 per cent sodium lauryl sulphate solutions of the adherent neutrophils in the dishes to arrive at the ct/minute per 0·2 absorbancy unit (AU) 280.

The effect of the α-globulins, synthetic esterase inhibitors, and other inhibitors on the HMPS activity of adherent neutrophil layers or on the random migration or chemotaxis of neutrophils in suspension was expressed as a percentage of the respective activity observed with cells preincubated in buffer alone but otherwise identically handled. Each experiment was performed at least three times with neutrophils from different normal donors on each occasion. Statistical analyses utilized the standard two sample Student's *t*-test.

RESULTS

INFLUENCE OF HUMAN PLASMA α-GLOBULINS ON NEUTROPHIL RANDOM MIGRATION

Preliminary experiments designed to detect leucocyte chemotactic activity in highly purified preparations of human α1-AT failed to reveal such activity, but instead showed that neutrophil migration was enhanced in the control chambers where α1-AT was present in both the cell and stimulus compartments. Since prolonged preincubation of α1-AT and neutrophils eliminated the enhancement of migration, the effect of variation of preincubation time was explored with a modified Boyden method and an 80 minute migration time. Neutrophils incubated with α1-AT for 1 minute, washed twice within a 2-minute period, and immediately loaded into migration chambers exhibited a two-fold increase in baseline random migration ($P = 0{\cdot}02$) (Fig. 1). Enhancement of random migration by α1-AT prepared by either method could no longer be appreciated with preincubation times longer than 5 minutes. Similar studies with purified α2-M detected a modest enhancement of random migration with a 1-minute preincubation, followed by slight

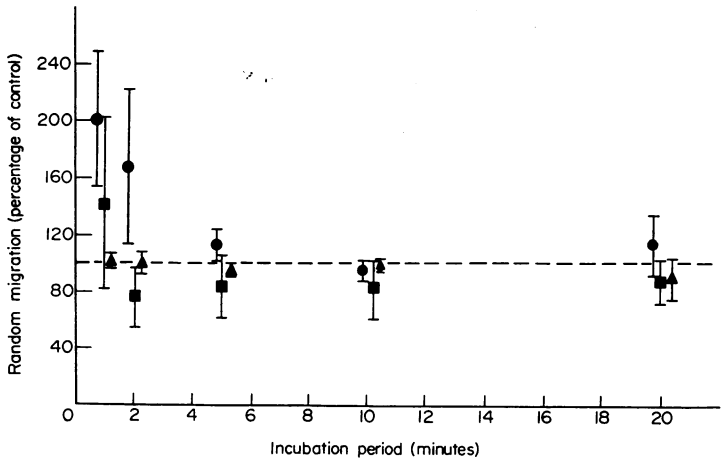

FIG. 1. Time course of α-globulin effects on human neutrophil random migration. Each point and bracket is the mean ± 1 s.d. for three experiments with different neutrophil donors in each experiment; control (100 per cent) migration ranged from nineteen to twenty-seven neutrophils/hpf. Protein concentrations were: α1-AT (●), 1·0 μg/ml; α2-M (■), 3·0 μg/ml; C1INH (▲), 2·0 μg/ml.

inhibition of random migration by 2 minutes, neither of which was statistically significant (Fig. 1). C1INH did not influence neutrophil random migration, nor did 1–20 μg/ml of soybean trypsin inhibitor (SBTI) or lima bean trypsin inhibitor (LBTI). Similar studies employing a radiomigration assay with a 150-minute migration time detected enhancement of neutrophil migration by α1-AT during the earliest 2 minutes, but revealed no effect of either α2-M or C1INH (Table 1).

INFLUENCE OF HUMAN PLASMA α-GLOBULINS ON NEUTROPHIL CHEMOTAXIS

The modified migration assays also allowed quantification of neutrophil chemotaxis in response to C3a or C5a after brief incubation periods. In the Boyden chambers, inhibition

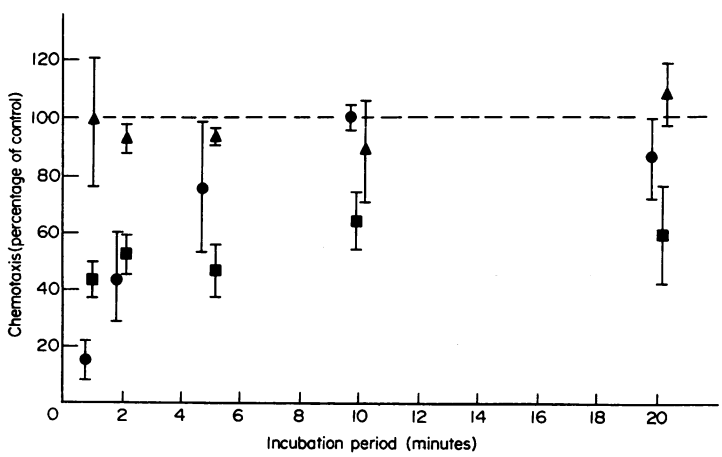

FIG. 2. Time course of α-globulin effects on human neutrophil chemotaxis. Each point and bracket is the mean ± 1 s.d. for three experiments with C5a as the stimulus in two experiments and C3a in one experiment; control (100 per cent) chemotaxis ranged from 43–96 neutrophils/hpf. Protein concentrations were: α1-AT (●), 1·0 μg/ml; α2-M (■), 3·0 μg/ml; C1INH (▲), 2·0 μg/ml.

of chemotaxis was significant after 1 ($P<0{\cdot}01$) or 2 ($P<0{\cdot}05$) minute exposures of neutrophils to α1-AT from either purification procedure, but was no longer seen after a 10-minute preincubation (Fig. 2). Exposure to α2-M for 1–20 minutes significantly ($P<0{\cdot}01$) inhibited neutrophil chemotaxis to C5a, while neither 1–20 μg/ml of SBTI or LBTI nor 2 μg/ml of CĪINH affected chemotactic migration. In the radiochemotactic assay, α1-AT again inhibited neutrophil chemotaxis to C5a after 1 or 2 minute exposures, α2-M was inhibitory after each preincubation period, and CĪINH had no effect on chemotaxis (Table 1). Parallel studies with C3a gave similar results in both assay systems.

TABLE 1

RADIOMIGRATION ASSAY OF ALPHA-GLOBULIN INHIBITOR EFFECTS ON HUMAN NEUTROPHIL MIGRATION

Agent	Incubation time (minutes)				
	1	2	5	10	20
Random migration*					
α1-AT (1·0 μg/ml)	124·5±23·3	117·8±3·7	101·6±6·4	95·5±12·0	96·1±5·2
α2-M (3·0 μg/ml)	89·3±7·8	102·1±12·7	90·6±24·3	106·2±14·9	79·8±12·6
CĪINH (2·0 μg/ml)	90·5±4·9	106·6±9·9	110·4±0·7	96·0±2·8	93·7±12·9
Chemotaxis†					
α1-AT (1·0 μg/ml)	44·6±14·2‡	53·0±5·7‡	93·3±9·9	78·5±9·2	83·4±2·8
α2-M (3·0 μg/ml)	60·5±22·6	42·1±16·9‡	57·2±12·7‡	60·5±21·9	47·3±19·8‡
CĪINH (2·0 μg/ml)	90·5±13·3	79·9±5·7	87·4±7·8	93·5±17·7	90·8±12·7

* Control (100 per cent) random migration ranged from 324–616 counts/4 minutes in three experiments (see Fig. 1).
† Control (100 per cent) chemotaxis ranged from 647–1013 net counts/4 minutes.
‡ Significant effect ($P\leqslant 0{\cdot}05$).

Analysis of the dose–response of α-globulin effects on human neutrophil migration in Boyden chambers revealed maximum enhancement of random migration and inhibition of chemotaxis at 1 μg/ml of α1-AT prepared by either method and 10 μg/ml of α2-M utilizing a 1-minute exposure period (Fig. 3). CĪINH from either purification procedure influenced neither random nor chemotactic migration up to a concentration of 10 μg/ml, but slightly enhanced chemotactic responsiveness at 100 μg/ml. Omitting the rapid cell washing step after preincubation did not influence the results.

EFFECT OF SYNTHETIC ESTERASE INHIBITORS ON HUMAN NEUTROPHIL MIGRATION

When isolated neutrophils were preincubated with either TPCK or TLCK for 2 or 30 minutes, washed twice in Hanks's solution, and resuspended in Medium 199–ovalbumin in Boyden migration chambers, their chemotactic response to either C5a (Fig. 4a) or C3a was inhibited in a dose-related manner. In contrast, the esterase inhibitors had opposite effects on random migration, with moderate enhancement by TLCK and inhibition by TPCK (Fig. 4b). Similar results were obtained with TPCK and TLCK in the radiomigration assay except that TLCK enhancement of random migration was appreciated only at the 10^{-6} M concentration (Table 2). The absence of a relationship of TLCK dose to enhancement of migration was not due to loss of neutrophil viability at the highest dose, since in parallel experiments trypan blue dye exclusion by neutrophils exposed to 10^{-4} M TLCK for 2 or 30 minutes was 98 and 96 per cent, respectively. Of all agents studied, only 10^{-4} M TPCK decreased neutrophil viability to 77 per cent and 48 per cent dye exclusion 1 hour after a 2-minute and a 30-minute preincubation, respectively.

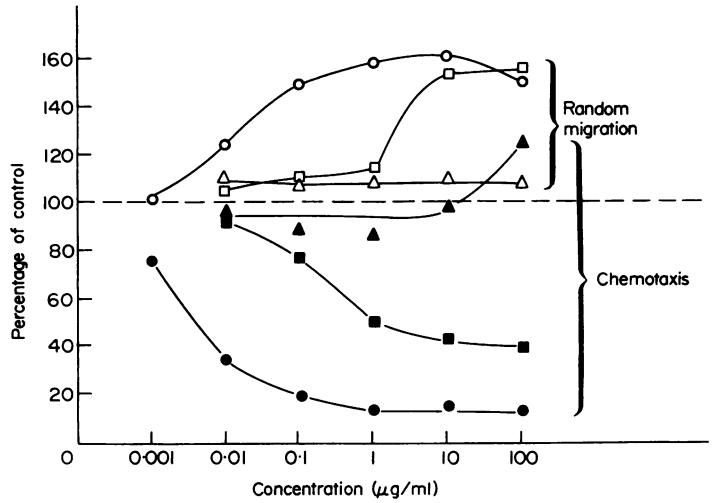

FIG. 3. Dose–response of α-globulin effects on human neutrophil migration. Each point is the mean of two experiments; mean control random migration (100 per cent) was twenty-six neutrophils/hpf and mean control chemotaxis to C5a (100 per cent) was 48 neutrophils/hpf. The exposure time was 1 minute at 25°. Proteins were: α1-AT (○, ●); α2-M (□, ■); CĪINH (△, ▲).

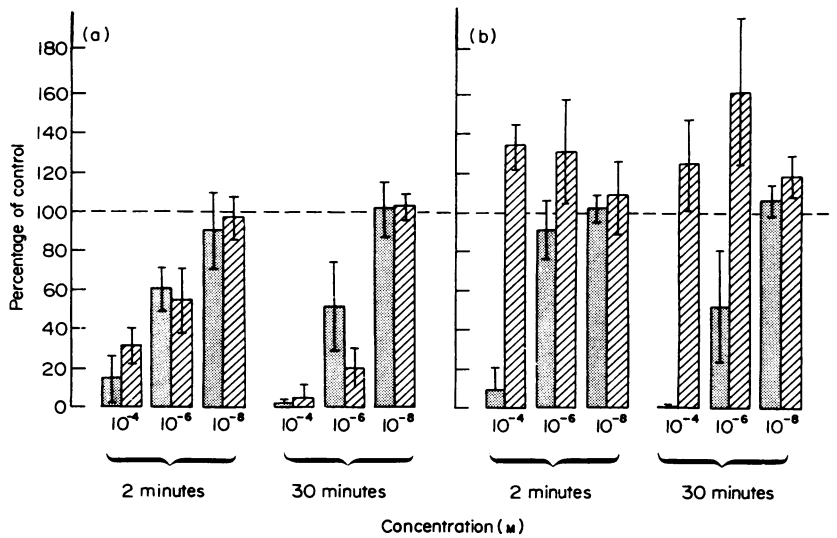

FIG. 4. Effect of synthetic esterase inhibitors on human neutrophil migration. Each column and bracket is the mean ± 1 s.d. of three experiments. (a) chemotaxis. Mean control (100 per cent) chemotaxis was fifty-nine neutrophils/hpf; C5a was the stimulus twice and C3a once. (b) Random migration. Mean control (100 per cent) random migration was twenty-three to forty-two neutrophils/hpf. The significance levels of TLCK effects on random migration were: 2 minutes, 10^{-4} M, $P<0{\cdot}01$; 30 minutes, 10^{-6} M, $P = 0{\cdot}05$; all others, $P>0{\cdot}05$. TPCK, stippled columns. TLCK, hatched columns.

CHARACTERISTICS OF ENHANCED NEUTROPHIL MIGRATION PRODUCED BY TLCK AND α-GLOBULINS

The finding that the presence of α1-AT, α2-M or TLCK on the cell side or both sides of the filter in Boyden chambers produced greater enhancement of random migration than

TABLE 2

RADIOMIGRATION ASSAY OF ESTERASE INHIBITOR EFFECTS ON HUMAN NEUTROPHIL MIGRATION

Agent	Incubation time (minutes)	
	2	30
Random migration*		
TLCK		
10^{-4} M	97·2	110·2
10^{-6} M	135·1	118·7
10^{-8} M	93·6	96·4
TPCK		
10^{-4} M	37·7	42·8
10^{-6} M	59·5	51·3
10^{-8} M	64·3	71·4
Chemotaxis†		
TLCK		
10^{-4} M	3·2	3·4
10^{-6} M	20·4	16·3
10^{-8} M	74·8	97·6
TPCK		
10^{-4} M	21·5	17·9
10^{-6} M	29·1	32·7
10^{-8} M	79·4	51·0

* Mean control (100 per cent) random migration was 669 counts/4 minutes in two experiments.

† Mean control (100 per cent) chemotaxis to C5a in two experiments was 499 net counts/4 minutes.

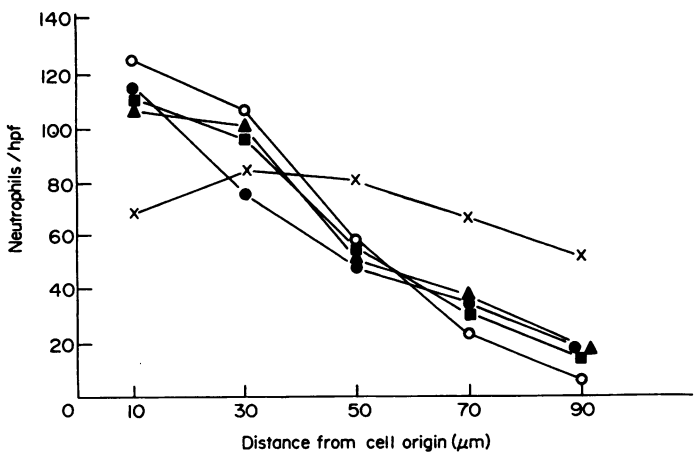

FIG. 5. Distribution of neutrophils within micropore filters. Concentrations of agents were: α1-AT (●), 1·0 μg/ml; α2-M (■), 10 μg/ml; TLCK (▲), 10^{-6} M. Preincubation exposure time was 2 minutes at 25°. Control (○). C5a (×).

the same concentration present only on the buffer side of the chamber suggested an effect directly on the cells. Both the enhancement of random migration and the inhibition of chemotaxis were unchanged when the pretreated cells were washed twice before assessment of migration, which indicated that each of the agents irreversibly influenced the neutrophils during the brief preincubation phase.

TABLE 3

INFLUENCE OF ALPHA-GLOBULINS AND ESTERASE INHIBITORS ON HUMAN NEUTROPHIL HEXOSE MONOPHOSPHATE SHUNT ACTIVITY

Agent	HMPS activity (percentage of control)*
α1-AT	
10 μg/ml	119·3 ± 12·6
1 μg/ml	114·0 ± 17·5
0·1 μg/ml	101·6 ± 4·8
0·01 μg/ml	100·3 ± 10·6
α2-M	
10 μg/ml	103·3 ± 16·6
1 μg/ml	103·5 ± 17·1
0·1 μg/ml	106·1 ± 12·7
0·01 μg/ml	101·4 ± 5·3
C1INH	
10 μg/ml	104·1 ± 16·9
1 μg/ml	108·3 ± 5·1
0·1 μg/ml	101·7 ± 7·3
0·01 μg/ml	103·4 ± 4·8
TPCK	
10^{-4} M	72·0 ± 10·4
10^{-6} M	103·2 ± 9·3
10^{-8} M	106·5 ± 4·9
TLCK	
10^{-4} M	117·1 ± 13·5
10^{-6} M	109·5 ± 12·1
10^{-8} M	102·6 ± 5·8
C5a†	238·4 ± 21·4

* Each value is the mean ± 1 s.d. for three experiments with 2 minute preincubation exposure times; HMPS control (100 per cent) had a range of 532–783 ct/minute/0·2 AU 280.

† Chemotactic activity was 146 neutrophils/hpf.

The distribution of neutrophils within micropore filters at the end of an incubation period was assessed in triplicate chambers to distinguish enhanced random migration from directed migration. In contrast to the effect of a chemotactic factor, C5a, which resulted in a peak cell density midway in the 100-μm thick filter, the agents tested gave a pattern characteristic of enhanced random migration with the highest cell concentration remaining at the origin and higher cell counts than the control in the opposite layers of the filter (Fig. 5). The effects on neutrophil HMPS activity of α-globulins, esterase inhibitors, and chemotactic factors were then compared (Table 3). Unlike C5a, which resulted in a doubling of HMPS activity of adherent neutrophils, α1-AT, α2-M, and TLCK only slightly stimulated HMPS activity at concentrations fully active on migration of neutrophil suspensions.

DISCUSSION

α-Globulin effects

Isolated washed human neutrophils exposed to low concentrations of purified human α1-AT or α2-M devoid of chemotactic activity exhibited transient enhancement of random migration (Fig. 1) and decreased responsiveness to chemotactic stimuli (Fig. 2) when assessed in Boyden chambers. Identical experiments assayed with a radiomigration method revealed similar chemotactic unresponsiveness, but enhancement of random migration

was not detectable with α2-M (Table 1). In the Boyden chamber assay, the effects on random migration and chemotaxis of neutrophils reached a plateau when the cells were preincubated with 1 μg/ml of α1-AT prepared by either method or 10 μg/ml of purified α2-M (Fig. 3). C1INH purified either by sequential column chromatography (Schreiber *et al.*, 1973b) or by immunoabsorption chromatography (Bing, personal communication) only slightly enhanced neutrophil chemotactic responsiveness at the highest concentration employed (100 μg/ml), and did not affect random migration. The limited effect of C1INH is consistent with a recent study which has documented stimulation of chemotaxis to C5a by C1INH at concentrations of 50–100 μg/ml with no influence on random migration (Smith, Hollers, Bing and Patrick, 1975).

The brief incubation periods afforded by the modified Boyden assay were critical to the recognition of the transient α1-AT effects on both random migration and chemotaxis. Our standard Boyden assay (Goetzl and Austen, 1972a) with incubation times 2·5-fold longer than those utilized in the present study detected only moderate inhibition of chemotaxis by α2-M, and no effect on random or directed migration by α1-AT. The importance of a brief assay period and the transient nature of the α1-AT effects, which are no longer detected with preincubation times exceeding 10 minutes, suggest that enhanced neutrophil random migration and chemotactic responsiveness have returned to baseline levels by 10 minutes after the interaction of the cells with α1-AT. Conversely, the enhancement of random migration by α1-AT and α2-M, and the chemotactic unresponsiveness generated by α1-AT which are quantified in 80 minute and 50 minute assays, respectively, actually seem to reflect events limited to the first 10 minutes of the assay period. The much longer incubation periods necessary for the radioassay (Goetzl and Austen, 1972b) may explain the less prominent results with α1-AT as well as the failure to detect α2-M enhancement of random migration (Table 1).

Synthetic esterase effects

Preincubation of isolated washed neutrophils with the synthetic esterase inhibitors TPCK or TLCK for either 2 minutes or 30 minutes in concentrations ranging from 10^{-8}–10^{-4} M resulted in a dose-related inhibition of chemotaxis in both Boyden and radiomigration assays (Fig. 4, Table 2). TPCK gave a concomitant inhibition of random migration over the same concentration range, while TLCK resulted in slight enhancement of random migration in both assays. The enhancement of random migration and suppression of chemotaxis by TLCK, which possesses specificity for trypsin-like esterases (Shaw, 1970), resembled the effect of α1-AT on neutrophils but persisted well past the 30 minute period of preincubation in contrast to the transient action of α1-AT (Figs 1, 2 and 4). Inhibition of both random migration and chemotaxis by TPCK, which is specific for chymotrypsin-like esterases (Shaw, 1970), is reminiscent of the action of the phosphonates or diisopropyl fluorophosphate, which inhibit migration (Goetzl and Austen, 1974) by inactivating the chymotrypsin-like esterase necessary for chemotaxis (Ward and Becker, 1968, 1970).

Mode of action of α-globulin and synthetic esterase inhibitors

The enhancement of neutrophil migration by α1-AT, α2-M and TLCK was a direct irreversible cell effect as evidenced by the greater response when the agents were present in the cell compartment of the migration chambers and the failure of washing after the exposure period to decrease the stimulation of cell migration. For the same reasons, the decreased chemotactic responsiveness which followed incubation of neutrophils with each agent was also an irreversible action on the cells. Assessment of the distribution of neutro-

phils within micropore filters by the method of Zigmond and Hirsch (Zigmond and Hirsch, 1973) after a migration period without a chemotactic stimulus showed that random migration was enhanced with no evidence for directed migration of the neutrophils (Fig. 5). This observation rules out a negative chemotactic effect by the inhibitors in the neutrophil compartment, but does not permit any conclusions as to the percentage of cells influenced by the inhibitors. These neutrophil responses also differ from those seen after the interaction of neutrophils with chemotactic factors to achieve unresponsiveness to other chemotactic stimuli, termed deactivation (Ward and Becker, 1968), or with ascorbate, which enhances both random and chemotactic migration of leucocytes (Goetzl, Wasserman, Gigli and Austen, 1974), since they are accompanied by only a modest stimulation of the neutrophil HMPS (Table 3). In contrast, ascorbate and diverse chemotactic factors result in a maximum 100–300 per cent increase in the activity of HMPS of adherent layers of human neutrophils (Goetzl and Austen, 1974; Goetzl *et al.*, 1974).

The inverse effects of α1-AT, α2-M and TLCK in enhancing random migration while inhibiting chemotaxis of human neutrophils in both kinetic and dose–response studies are consistent with binding to and activation by these agents of neutrophil surface receptors which also can interact with chemotactic factors. Several enzymes which have been found in or on the surface of leucocytes are susceptible to inactivation by the α-globulins and presumably bind these proteins. α1-AT inhibits the spontaneous fibrinolytic enzyme of human neutrophils, and the lysosomal neutral protease and elastase of neutrophils which are also inhibited by α2-M (Janoff, 1972). More recently, we have described a human neutrophil membrane-associated protease which can generate a neutral peptide mediator of inflammation when either intact cells or cell extracts cleave a plasma protein substrate, and which is inhibited by microgram quantities of α1-AT but not by α2-M (Wintroub *et al.*, 1974). Unoccupied enzyme active sites or other receptors on the neutrophil surface presumably are either liberated during washing and isolation of cells or are generated *de novo* as part of the membrane restorative process (Smolen and Shohet, 1974). These receptors may bind newly added α1-AT, α2-M, or TLCK, resulting in both transient stimulation of random migration and a period of decreased chemotactic responsiveness with a duration related to the efficiency of processing of the occupied site by the cell. The formation of an α2-M–receptor complex on the surface of a neutrophil may provide the same stimulus to random migration as an α1-AT–receptor complex, but the persistent chemotactic inhibition produced by α2-M would imply that a longer period is required for processing of the larger α2-M–receptor complex. The greater potency of α1–AT than α2–M in influencing neutrophil migration is similar to the 10–30-fold greater inhibition of trypsin by α1-AT than α2-M on a weight basis (Vogel, Trautschold and Werle, 1968). The similar effect to that of α1-AT on neutrophil migration produced by TLCK, which preferentially binds to trypsin-like esterases (Shaw, 1970), suggests that the sites responsible for the dual stimulation of random migration and inhibition of chemotaxis may be trypsin-like in their specificity. In contrast, the suppression of both random and directed migration by TPCK, which selectively inhibits chymotrypsin-like esterases (Shaw, 1970), suggests that TPCK binds to and blocks the previously described activated and chemotactic factor-activatable esterases which are essential to polymorphonuclear leucocyte migration and are similar to chymotrypsin in their site specificity (Ward and Becker, 1968, 1970). Fluorescent antibody studies have shown α1-AT bound in and on the surface of some leucocytes, which probably represented adsorbed plasma α1-AT since the density of staining varied with the plasma concentration of α1-AT of the cell donor (Cohen, 1973).

The influences of α1-AT and α2-M on neutrophil migration imply that such surface α-globulins also may be present on human neutrophils and could influence their migration and response to chemotactic stimuli.

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