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Diverging effects of chemotactic serum peptides and synthetic f-Met-Leu-Phe on neutrophil locomotion and adhesion

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Summary. The chemotactic serum peptide preparations CAT 1.6.1. and $C5a_{des\ Arg}$ induced marked directional locomotion over a wide concentration range without significant effects on random locomotion and adhesion of human neutrophils in Gey's solution containing 2% HSA. In contrast, f-Met-Leu-Phe produced marked negative chemokinetic effects and its capacity to induce directional locomotion was more limited with respect to magnitude and concentration range. The negative chemokinetic effect of f-Met-Leu-Phe correlated closely with increased spreading and cell adhesion.

INTRODUCTION

Several studies suggest that chemotactic activity is always associated with chemokinesis, increased adhesion to the substratum, cell aggregation, release of lysosomal enzymes *in vitro* and neutropenia *in vivo* (Fehr & Jacob, 1977; Smith, Hollers, Patrick & Hassett, 1979a; Kreutzer, O'Flaherty, Orr, Showell, Ward & Becker, 1978). These findings have been used to construct unifying concepts for these different biological activities. Other studies seem to question this idea by demonstrating that marked chemotaxis occurs without effects on adhesion to the substratum and chemokinesis (Keller, Wissler, Hess & Cottier, 1978) or that adhesion is decreased (Smith, Lackie & Wilkinson, 1979b). The present study further queries the unifying concepts by showing that different cytotaxins produce markedly diverging effects on neutrophil locomotion and adhesion.

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MATERIALS AND METHODS

Materials

Gey's solution containing 2% human serum albumin (HSA) was used as basic medium. HSA was obtained from Behringwerke Marburg (FRG), the synthetic peptide f-Met-Leu-Phe (FMLP) was a gift of Dr Peter Dukor, Ciba-Geigy AG, Basle, Switzerland. The same dose response curves were also observed with a FMLP preparation that was kindly provided by Dr R. Snyderman (Durham, U.S.A.) and independently by one of us (B.D.) using a preparation obtained from Dr E. Wünsch (Martinsried, Germany). Fresh dilutions were made for each experiment from stock solutions (10^{-2} M in DMSO) which were either prepared on the same day or kept at $-20°$ for no longer than 2 weeks. Purified chemotactic serum peptides containing classi-

cal anaphylatoxin (CAT 1.6.1.) were prepared from dextran-activated pig serum (average MW 8700, Wissler, 1972). $C5a_{des\ Arg}$ (MW 9000) was isolated from hog serum as described by Grossklaus, Damerau, Lemgo & Vogt (1976). The concentration of CAT 1.6.1. was determined spectrophotometrically (Keller *et al.*, 1978; Wissler, 1972), that of $C5a_{des\ Arg}$ or FMLP by dry weight.

Preparation of neutrophil granulocytes

Neutrophil granulocytes from peripheral human blood were prepared by means of Isopaque–Methocel. In some experiments (direct observation) leucocytes were further purified by passage through a Ficoll–sodium metrizoate mixture (Lymphoprep; Nyegaard & Co., AS, Oslo) in order to remove lymphocytes and monocytes (Böyum, 1968).

Assessment of neutrophil adhesion, random and directional locomotion

Neutrophil random and directional locomotion was determined in triplicate by means of the two filter count method (Keller, Gerber, Hess & Cottier, 1976) using an incubation time of 3 h or 1 h, respectively. At optimal concentrations all cytotaxins produced marked orientation (about 90%) as assessed in the visual assay described by Zigmond (1977). Furthermore, locomotion and adhesion was studied by direct observation in Sykes-Moore chambers using phase-contrast microscopy and reflection-contrast microscopy (Keller, Barandum, Kistler & Ploem, 1979). Adhesion was also tested by determining the proportion of neutrophils which remain attached after shaking of the chamber-slides (Lab-tek, Naperville, Ill.) on a Vortex-type mixer (Keller *et al.*, 1979).

RESULTS

Effects of CAT 1.6.1., $C5a_{des\ Arg}$ and FMLP on random and directional locomotion of neutrophils

The threshold for the response to CAT 1.6.1., $C5a_{des\ Arg}$ and FMLP was found to be in the order of 10^{-9}–10^{-8} M. In the experiment represented in Fig. 1a FMLP produced a two-fold increment over control values at a concentration of 10^{-9} M, a four-fold increment at $5 \cdot 10^{-9}$ M and as much as 22-fold at 10^{-8} M. All three cytotaxins induced a dose-dependent increase of neutrophil accumulation *in vitro*. Their maximal effects, however, differed considerably. FMLP produced less neutrophil accumulation than the other peptides. No significant differences in the maximum response to CAT 1.6.1. and $C5a_{des\ Arg}$ was observed in tests performed with leucocytes from the same preparation (not shown). Higher concentrations of all peptides lead to a decrease of leucocyte migration which was partial for CAT 1.6.1. and $C5a_{des\ Arg}$ but complete for FMLP (Fig. 1a). Consequently, the two preparations of serum peptides produced neutrophil accumulation over a much broader concentration range.

The results of filter experiments performed in absence of a gradient (random locomotion) are recorded in Fig. 1b. Similar results were obtained by direct observation in Sykes-Moore chambers. In comparison to the controls (Gey's solution containing 2% HSA), FMLP produced a dose-dependent inhibition of random locomotion at concentrations higher than 10^{-8} M, resulting in almost total arrest of locomotion at 10^{-5} M. CAT 1.6.1. or $C5a_{des\ Arg}$ produced no such inhibition. Using $C5a_{des\ Arg}$ one might even suspect a slight stimulation (10^{-8}–$3 \cdot 10^{-7}$ M) but this was not statistically significant.

Effects on neutrophil adhesion

In contrast to CAT 1.6.1., the synthetic peptide FMLP produced increased neutrophil adhesion at chemotactic concentrations as judged by the proportion of neutrophils sticking to glass (Fig. 1c). Increased adhesion to the substratum parallels the negative chemokinetic effect of FMLP (Fig. 1b).

The findings were confirmed and extended by means of reflection-contrast microscopy. A dose-dependent increase of adhesion was observed with FMLP. No obvious changes were observed at concentrations of 10^{-14}, 10^{-12} and 10^{-10} M. Grey areas of contact were found to be increased in size and intensity at a concentration of 10^{-8} M. Furthermore, there was formation of very long retraction fibres which tended to break when the cells were moving on (Fig. 2E). At a FMLP concentration of 10^{-6} M and higher, neutrophils became immobilized and showed increased spreading. Large black areas at the cell periphery were detected by reflection-contrast microscopy (Fig. 2F). CAT 1.6.1. or $C5a_{des\ Arg}$ produced no such marked changes of the reflection-contrast picture obtained with Gey's solution containing 2% HSA only. The occurrence of less conspicuous effects such as smaller changes in the number of retraction fibres is being investigated.

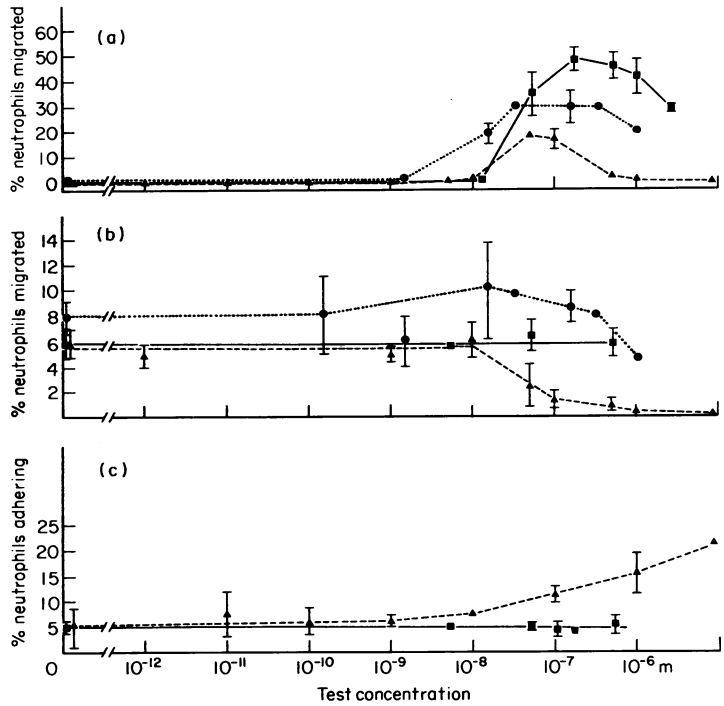

Fig. 1. Directional locomotion (a), random locomotion (b) and adhesion to glass (c) of neutrophils responding to CAT 1.6.1. (■——■), $C5a_{des\ Arg}$ (●·····●) or FMLP (▲------▲) in Gey's solution containing 2% HSA was determined (mean ± SD). The response to CAT 1.6.1. and FMLP was tested with cells from the same suspension, whereas the results for $C5a_{des\ Arg}$ were obtained with a different batch of cells. The incubation time for experiments a and b was 1 and 3 h, respectively.

DISCUSSION

Adhesion and chemokinesis depend on a variety of environmental factors (Keller *et al.*, 1979) and the respective effects of chemicals may vary according to the environmental conditions. Therefore, any statements on results including those which follow are restricted to the test conditions used. Under the control conditions chosen for the present experiments neutrophils exhibit marked locomotion and relatively weak adhesion (Keller *et al.*, 1979).

Diverging reports concerning the effects of cytotaxins on neutrophil adhesion and chemokinesis have been published by several investigators using different test conditions (Fehr & Jacob, 1977; Smith *et al.*, 1979a; Smith *et al.*, 1979b). The present study showed that cytotaxins can exhibit markedly diverging biological effects under identical test conditions. In contrast to CAT 1.6.1. and $C5a_{des\ Arg}$, the synthetic peptide FMLP had chemotactic activity as well as marked negative chemokinetic effects associated with increased neutrophil adhesion. High concentrations of FMLP annihilated the positive chemokinetic effect and the decreased adhesion to the substratum induced by the addition of 2% HSA to Gey's solution. The neutrophils in Gey's solution containing 2% HSA became anchored similar to cells in Gey's solution only (Keller *et al.*, 1978, 1979). Therefore, random as well as directional locomotion were decreased. The findings showed a close correlation between chemokinesis and adhesion, but there was no evidence for a relationship between chemotactic activity and changes in adhesiveness. Diverging observations concerning the relation between adhesion and chemotaxis reached by other investigators (Fehr & Jacob, 1977; Smith *et al.*, 1979b; O'Flaherty, Kreutzer & Ward, 1978) are compatible with this interpretation.

The results showed that serum peptides are on a molar basis at least as active as FMLP. Furthermore, they are much more effective in producing leucocyte

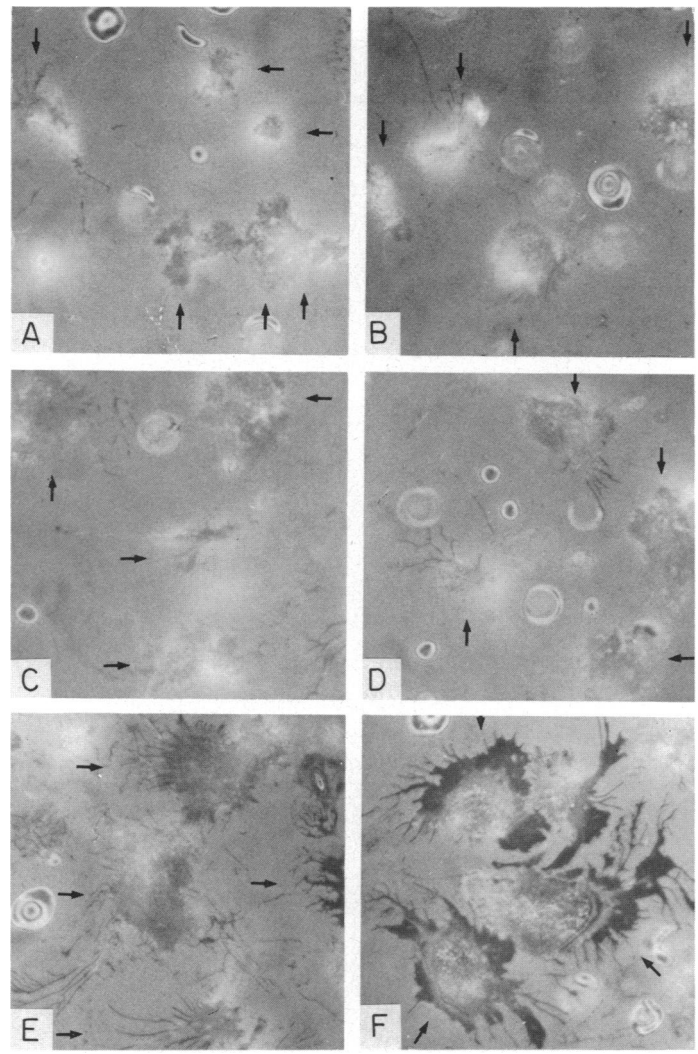

Fig. 2. Interaction of neutrophils with the substratum (glass) was studied by means of reflection-contrast microscopy (700 ×). The following cytotoxins were added to Gey's solution containing 2% HSA: (A) none (control); (B) $C5a_{des\ Arg}$ ($1{\cdot}1 \times 10^{-6}$M); (C) CAT 1.6.1. ($2{\cdot}6 \times 10^{-6}$M); (D) CAT 1.6.1. ($5{\cdot}3 \times 10^{-7}$M); (E) FMLP (10^{-8}M) (F) FMLP (10^{-5}M). Photographs were taken at 30 min of incubation. The position of individual leucocytes is indicated by arrows (→).

accumulation at the optimal dose as compared to the synthetic peptide.

FMLP and related synthetic peptides have been used as model chemotactic factors to study a wide variety of biological and biochemical reactions which are believed to be strictly associated with chemotaxis. Our results suggest that highly purified serum peptides act more specifically than synthetic FMLP, at least under the test conditions used. Several reports suggest that FMLP and related synthetic peptides promote also neutrophil aggregation, release of lysosomal enzymes, metabolic burst, neutropenia and other reactions (Fehr & Jacob, 1977; Kreutzer *et al.*, 1978; Becker, Sigman & Oliver, 1979). Accordingly, unifying concepts associating all different activities to chemotactic stimulation have been developed (Kreutzer *et al.*, 1978; Naccache, Showell, Becker & Sha'Afi, 1977). The findings that highly chemotactic CAT 1.6.1. or $C5a_{des\ Arg}$ have no significant chemokinetic effects and fail to change adhesion in our experimen-

tal system at chemotactic concentrations, shows that the fascinating concepts may have certain limitations. It is conceivable that some reactions such as cell aggregation, release of lysosomal enzymes *in vitro* and neutropenia *in vivo* are related to chemokinesis and neutrophil adhesion rather than chemotaxis. There is a lot of evidence suggesting that increased contact with the substratum results in release of lysosomal enzymes and a metabolic burst. Dissociation between release of lysosomal enzymes and chemotaxis has been reported (Spilberg, Mandell, Metha, Sullivan & Simchowitz, 1978). There is, however, a striking correlation between the dose-dependent increase of neutrophil adhesion in response to f-Met-Leu-Phe found in the present experiments and lysozyme release as measured by Cramer & Gallin (1979).

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