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CHEMOTACTIC FACTOR BINDING BY METASTATIC TUMOUR CELLS: EVIDENCE FOR A FORMYL-PEPTIDE RECEPTOR ON A NON-MYELOGENOUS CELL

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SUMMARY

Analysis of fMet-Leu-[^{3}H]Phe binding to Walker 256 carcinosarcoma cells demonstrated both saturable and reversible binding, and indicated the presence of a single population of binding sites having an equilibrium dissociation constant: $K_D = 15{\cdot}7 \pm 3{\cdot}3 \times 10^{-9}$ M, and with 2425 ± 204 binding sites per cell. The specificity of the binding site was investigated by competitive inhibition of fMet-Leu-[^{3}H]Phe binding studies using 10 oligoformyl peptides. These results demonstrated an order of peptide reactivity with marked similarity in specificity to the leucocyte binding sites for the formyl-peptides. The most active peptides also had potent agonist activity as determined by their ability to increase the cells' adherence response to nylon-wool fibres. In addition, a competitive antagonist of the formyl-peptide receptor, tert-butoxy-Phe-Leu-Phe-Leu-Phe, completely abolished the adherence response induced by fMet-Leu-Phe, but had no inhibitory effect on the adherence response caused by the tumour-promoting agent, phorbol myristate acetate.

These data demonstrate that formyl-peptide receptors may be more common than we have anticipated and may be found on cells not derived from the myeloid series. Furthermore, these studies advance our understanding of stimulus-coupled responses in tumour cells.

INTRODUCTION

The NH_2-formylated peptides elicit a number of responses in leucocytes. These include chemotaxis, chemokinesis, exocytosis, cell-to-substrate adherence, cell-to-cell aggregation and changes in Ca^{2+} levels within the cytosol of the cells (Schiffmann, Corcoran & Wahl, 1975; Hsu & Becker, 1975; Becker, 1979; Becker, Showell, Naccache & Sha'afi, 1981; Naccache *et al.* 1979*a*,*b*; O'Flaherty, Kreutzer & Ward, 1978). Previous studies in our laboratory have shown that the Walker 256 carcinosarcoma cells are also capable of responding to the formyl peptides *in vitro*. Responses that have been identified include migration in the Boyden chamber assay, increased adherence to foreign surfaces and cell swelling (Wass *et al.* 1981; Varani, Wass, Piontek & Ward, 1981; Lam *et al.* 1981; Varani, 1982; Fantone, Elgas, Weinberger & Varani, 1983*a*). Additionally, it has been shown that the intraperitoneal injection of the peptide, fMet-Leu-Phe, into rats bearing circulating Walker

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cells, induces localization of the tumour cells in the peritoneal mesenteries and the subsequent formation of tumours at these sites (Orr *et al.* 1981).

It is well established that the leucocyte response to the NH_2-formylated peptides follows from the binding of the ligand to high-affinity receptors on the cell surface (Becker, 1979). The similarities between the responses of Walker carcinosarcoma cells and leucocytes suggests that there are also receptors for the NH_2-formylated peptides on the Walker cells. However, the binding of these peptides by the Walker cells has not been formally characterized. In this report, we characterize the binding of fMet-Leu-[^{3}H]Phe to the Walker carcinosarcoma cells and correlate biological responses with peptide binding.

MATERIALS AND METHODS

Chemicals

The synthetic peptides used in this study are fMet, D,L-methionine, fMet-Phe, fMet-Leu-Phe and carbobenzoxy(CBZ)-Phe-Met, Sigma Chemical Co., St Louis, MO; fMet-Met-Phe, Miles-Yeda, Ltd, Rehovot, Israel; fMet-Met-Met, United States Biochemical Corp., Cleveland, Ohio; fMet-Phe-Met and tert-butoxy(t-boc)-Phe-Leu-Phe-Leu-Phe, Peninsula Laboratories, Inc., San Carlos, Ca.; fMet-Leu, Met-Leu-Phe and fMet-Leu-Phe-Phe were generous gifts from Dr Richard J. Freer, Department of Pharmacology, Medical College of Virginia. The biological activity of these peptides for rabbit neutrophils (Showell *et al.* 1976; Freer *et al.* 1980, 1982; Marasco, Showell, Freer & Becker, 1982*c*) and rat (Marasco, Fantone, Freer & Ward, 1983) has been reported elsewhere. The radiolabelled peptide, fMet-Leu-[^{3}H]Phe (47·6 Ci/mmol) was obtained from New England Nuclear; Boston, MA. Safety-Solve scintillation fluid was purchased from Research Products International. 2-Deoxyglucose and phorbol myristate acetate (PMA) were obtained from Sigma Chemical Co.

Cells

The Walker 256 carcinosarcoma cells used in this study were originally derived from a rat mammary tumour and have been maintained for several years by serial passage in rats and in culture (Spiro & Mundy, 1980). These cells have been shown previously to respond to chemotactic factors, including fMet-Leu-Phe, in the Boyden chamber assay (Lam *et al.* 1981), in the foreign-surface adherence assay (Varani *et al.* 1981) and in the cell swelling assay (Wass *et al.* 1981). The cells were grown in suspension culture using RPMI-1640 medium supplemented with 10% (v/v) foetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin as the growth medium. They were grown at 37°C in 5% CO_2/95% air. Before use, the cells were determined to be free of mycoplasma by growth on mycoplasma agar and growth in mycoplasma broth.

Measurement of fMet-Leu-[^{3}H]Phe binding

Binding studies were carried out in Na^+-free, high-K^+ modified Krebs buffer of the following composition: [K^+],157 mM;[Cl^-],130 mM;[PO_4^{3-}],16·6mM;[Mg^{2+}],1·22mM;[$CaCl_2$],0·5mM; [KCN],1mM; and [cytochalasin β],5 μg/ml; pH 7·4; 10 mM-2-deoxyglucose was included in the buffer to minimize internalization (Marasco *et al.* 1983; Niedel, Kahane & Cuatrecasas, 1979*a*). This buffer has been recently shown by DeTogni *et al.* (1983) to increase both human neutrophil formyl-peptide receptor number and specific affinity for fMet-Leu-[^{3}H]Phe.

The cultured cells were washed twice with the incubation buffer, incubated at 37°C for 5 min, washed again, and allowed to stand at room temperature (24°C) for 30 min before initiating the binding study. The binding studies were performed by incubating 100 μl of cells (5×10^7 cells/ml) and 5 μl of radiolabelled peptide in a siliconized 12 mm × 75 mm glass test tube. After 45 min (unless otherwise specified), 2 ml of modified Krebs buffer (4°C) were added to the tube and vortexed. The cells were then harvested by a glass-fibre vacuum filtration method (Marasco *et al.*

1983; Fantone *et al.* 1983*b*). The filters were washed with an additional 8 ml of buffer, dried and cell-bound radioactivity was measured by liquid scintillation counting. Non-saturable binding was defined as the amount of fMet-Leu-[^{3}H]Phe bound in the presence of a 1000-fold molar excess of non-radiolabelled fMet-Leu-Phe. All data points were determined in triplicate, and the standard error of the mean was consistently less than ±5%.

For dissociation experiments, the reversibility of fMet-Leu-[^{3}H]Phe binding was determined by the addition of a 1000-fold molar excess of unlabelled fMet-Leu-Phe after the plateau of binding had been reached. At various times thereafter, cells were harvested and assayed for loss of cell-bound peptide. All data points were determined in triplicate.

The specificity of the formyl-peptide binding site was determined by inhibition of fMet-Leu-[^{3}H]Phe binding. In brief, 5×10^6 tumour cells and fMet-Leu-[^{3}H]Phe (50 nM) were incubated for 30 min in the presence of five 10-fold dilutions of the numerous different oligopeptides listed (see *Chemicals*, above). Competitive inhibition curves were generated and ID_{50} values (concentration of unlabelled peptide yielding 50% of maximal inhibition) were tabulated. We also determined structure activity relationships using the adherence assay to confirm these experiments further.

The binding data were analysed with NONLIN, a weighted non-linear least-squares regression analysis computer program (Metzler, 1969; Fischel & Medzihradsky, 1981; Marasco, Feltner & Ward, 1984*a*). Non-specific binding was first subtracted from total binding either at individual points or as a parameter. Similar results were obtained using both methods. The weighted-sums of square deviations (WSSD) generated by NONLIN for 1 saturable, 2 saturable and 2 saturable + 1 non-saturable component models were compared using the F-test (Boxenbaum, Riegelman & Glashoff, 1974; Sokal & Rohlf, 1973). The weighting factor was $1/y$ obs.

Adherence assay

Adherence to nylon fibres was used as the indicator of the biological response to the peptides. The assay was performed at 24°C and the procedure used was very similar to that originally described by MacGregor, Spagnuolo & Lentnek (1974). A 100 mg sample of scrubbed nylon fibres (Associated Biomedic Systems; Buffalo, N.Y.) was packed into plastic 10 cm^3 syringes (to the 3 cm^3 mark) and placed vertically in a test-tube rack. The columns were prewashed by running through 10 ml of the reaction medium (RPMI-1640 medium with 10% foetal bovine serum). After prewashing the columns, 2 ml samples of untreated cells (2×10^6 cells) or cells treated at various times with an appropriate dose of the stimulating agent were added to duplicate columns and allowed to adhere for 3 min. The non-attached cells were then washed through with 20 ml of phosphate-buffered saline and counted. All cell counts were made using a Coulter Counter, model ZB (Coulter Diagnostics; Hialeah, Florida) and from this the percentage of adhering cells was determined. A complete description of the assay procedure can be found in our recent reports (Varani *et al.* 1981; Fantone *et al.* 1983*a*). In most of the experiments, fMet-Leu-Phe was used as the stimulating agent. However, phorbol myristate acetate (PMA) was used in some studies. When this agent was used, the assay was run in exactly the same way except that the cells were treated with the agent 5 min before their addition to the columns.

For the studies examining the inhibition of either the fMet-Leu-Phe- or PMA-induced adherence by competitive antagonists of the formyl-peptides (O'Flaherty *et al.* 1978*b*), the following protocol was used. Cells were counted and then divided into two pools. One pool was treated with control buffer and the other was treated with the appropriate amount of inhibitor to give the desired final concentration (normally, 20 µl of a 100× solution per 2 ml). The two pools of cells were each immediately subdivided into two pools. One group of each pair was used as a control; the second group of each pair was stimulated with the appropriate agent. The nylon-adherence assay was then run in the normal manner.

RESULTS

Time course of fMet-Leu-[^{3}H]Phe binding

The kinetics of fMet-Leu-[^{3}H]Phe binding were investigated by incubating Walker cells and fMet-Leu-[^{3}H]Phe (13·4 nM) for various periods of time at 24°C.

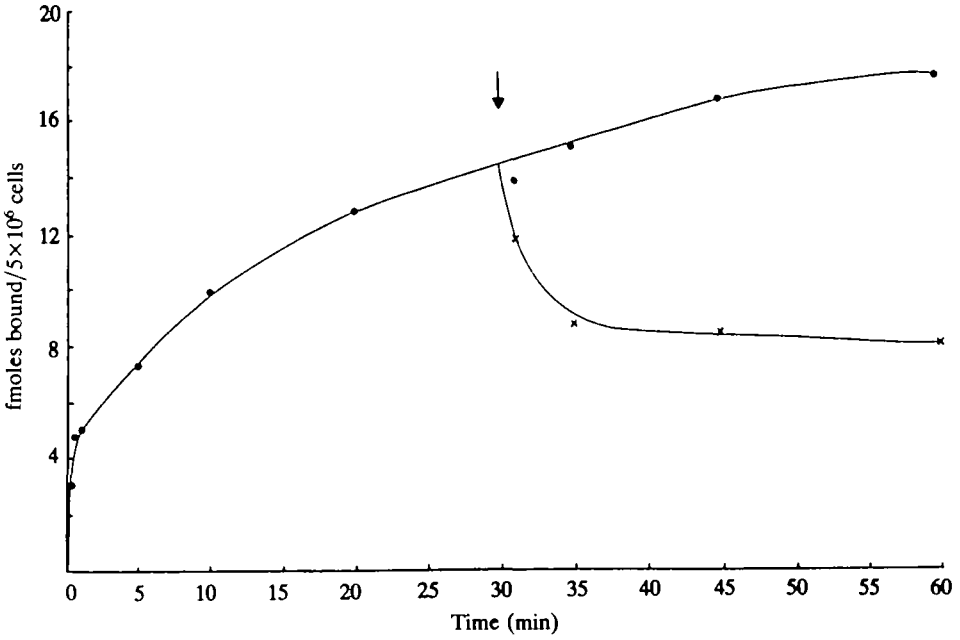

Fig. 1. Time-course and reversibility of fMet-Leu-[^{3}H]Phe binding to Walker carcinosarcoma cells. fMet-Leu-[^{3}H]Phe (13·4 nM) was incubated with 5×10^6 Walker cells at 24°C and the binding was measured at the various time intervals indicated (●——●). At 30 min (arrow), a 1000-fold excess of unlabelled fMet-Leu-Phe was added to some incubation mixtures (×——×). At various time-points thereafter, a sample of cells was removed, washed, rapidly filtered and counted. Each point is the mean of triplicate determinations and individual points varied less than ±10% of the mean.

The binding of fMet-Leu-[^{3}H]Phe reached equilibrium within 45 min (Fig. 1) and the plateau was maintained throughout a 90 min incubation (data not shown). The reversibility of binding of fMet-Leu-[^{3}H]Phe to the surface receptor on the Walker cell was tested by adding a 1000-fold excess (13·4 μM) of unlabelled fMet-Leu-Phe to some incubation mixtures after equilibrium was reached. As shown in Fig. 1, the binding was reversible with 55% of the labelled peptide being dissociated after 30 min.

Saturability of fMet-Leu-[^{3}H]Phe binding

To determine if the non-myelogenous Walker carcinosarcoma cells had saturable binding sites for fMet-Leu-Phe, the cells were incubated with various concentrations of fMet-Leu-[^{3}H]Phe in the presence or absence of a 1000-fold excess of unlabelled fMet-Leu-Phe and analysed for fMet-Leu-[^{3}H]Phe binding using the rapid filtration assay. As seen in Fig. 2, saturation of formyl-peptide binding sites occurred at high concentrations of fMet-Leu-[^{3}H]Phe (i.e. 60 nM). Non-specific binding was linear over the entire range of fMet-Leu-[^{3}H]Phe concentrations tested (data not shown).

The number of fMet-Leu-[^{3}H]Phe binding sites per cell and the dissociation constant (K_D) were determined by Scatchard (1949) analysis of the data shown in

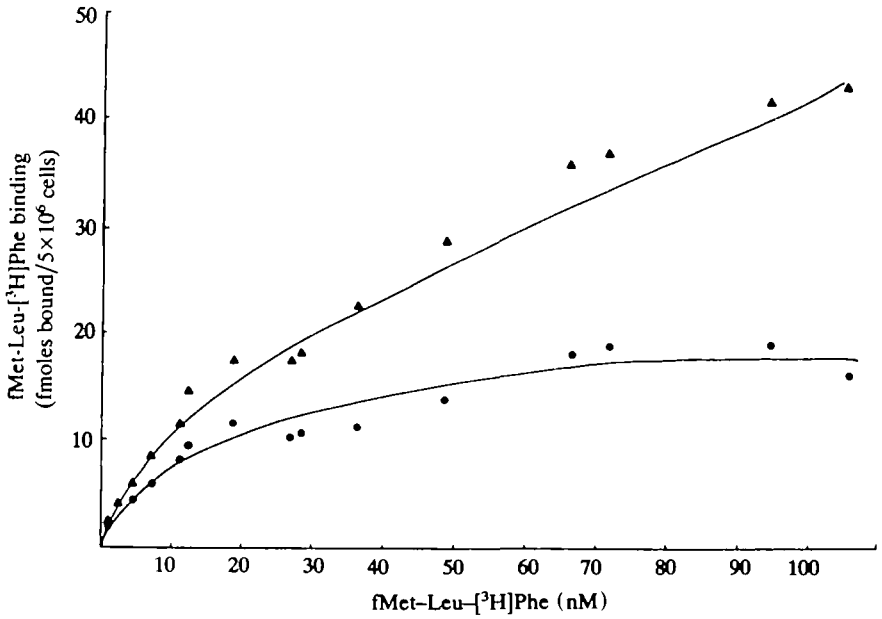

Fig. 2. Total (▲) and specific (●) binding of fMet-Leu-[^{3}H]Phe to Walker carcinosarcoma cells as a function of fMet-Leu-[^{3}H]Phe concentration. fMet-Leu-[^{3}H]Phe was incubated at the given concentration for 45 min at 24°C with 5×10^6 cells and the specific binding was calculated. Each point is the mean of triplicate determinations and individual points varied less than ±10% of the mean.

Fig. 2 (Fig. 3). As can be seen, a linear relationship was obtained. This demonstrates that the Walker carcinosarcoma cells have a single class of non-interacting binding sites for the formyl-peptide with a dissociation constant (K_D) of $15{\cdot}7 \pm 3{\cdot}3\times10^{-9}$ M ($n = 2$) (mean ± S.E.M) and that there are 2425 ± 204 binding sites per cell. It should be noted that in certain experiments we used Hanks' buffer rather than the Na^+-free, high-K^+ modified Krebs buffer. Very similar results to those presented here were obtained.

Kinetics of the adherence response induced by fMet-Leu-Phe

One of the several criteria used as evidence for the presence of a receptor for the formyl-peptides on the leucocyte plasma membrane is that the kinetics of peptide binding should be in accord with the kinetics of biological responsiveness induced by these same peptides (Becker, 1979). In the present study, we observed a similar association between fMet-Leu-[^{3}H]Phe binding to the Walker cells and the adherence response induced by fMet-Leu-Phe. As shown in Fig. 4, 10^{-6} M-fMet-Leu-Phe induced a rapid, transient adherence response. The maximum response, representing approximately 15% of the total population, occurred when the cells were added to the column within 5 min after stimulation. By 15–20 min after stimulation, the ability to respond was ablated.

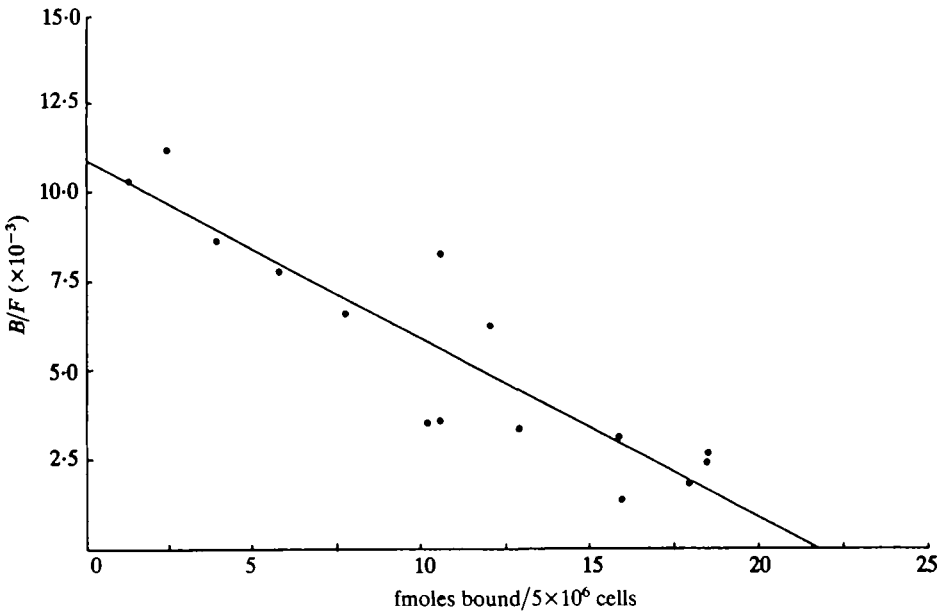

Fig. 3. Scatchard analysis of the specific fMet-Leu-[^{3}H]Phe binding to Walker carcinosarcoma cells. Free ligand (F) is the amount of fMet-Leu-[^{3}H]Phe added minus the total fMet-Leu-[^{3}H]Phe binding (B). The equilibrium dissociation constant (K_D) and the number of binding sites per cell were calculated from the slope and x-intercept, respectively. The correlation coefficient $r = 0{\cdot}90$.

In contrast, the adherence induced by the tumour promotor PMA (250 ng/ml) required 10–15 min after treatment before the maximal adherence response was seen. Although the response to PMA was also transient, the time-course was broader than the response seen with fMet-Leu-Phe. In addition, a greater percentage of cells responded to PMA than to fMet-Leu-Phe. Whether the differences in the percentages of responding cells induced by fMet-Leu-Phe and PMA indicate the presence of multiple subpopulations of cells with receptors for different agents or are due to heterogeneity in the stage of cellular differentiation is not known. It is quite evident, however, that both the time-course and degree of responsiveness are different for these two agents and are in agreement with temporal differences in several biological responses to these agents seen with leucocytes (DeChatelet, Shinley & Johnston, 1976).

Specificity of binding sites for N-*formylated peptides*

The specificity of the binding site was first determined by the ability of structurally related formyl-peptides to compete, with varying degrees of efficiency, with fMet-Leu-[^{3}H]Phe for binding to the putative receptor. As can be seen in Table 1, a greater than 200-fold range of binding activities is seen for peptides modified in one or another of the proposed first five critical areas of peptide–receptor interaction (Freer *et al.* 1980, 1982; Marasco *et al.* 1982*c*; Marasco *et al.* 1983). In addition, the

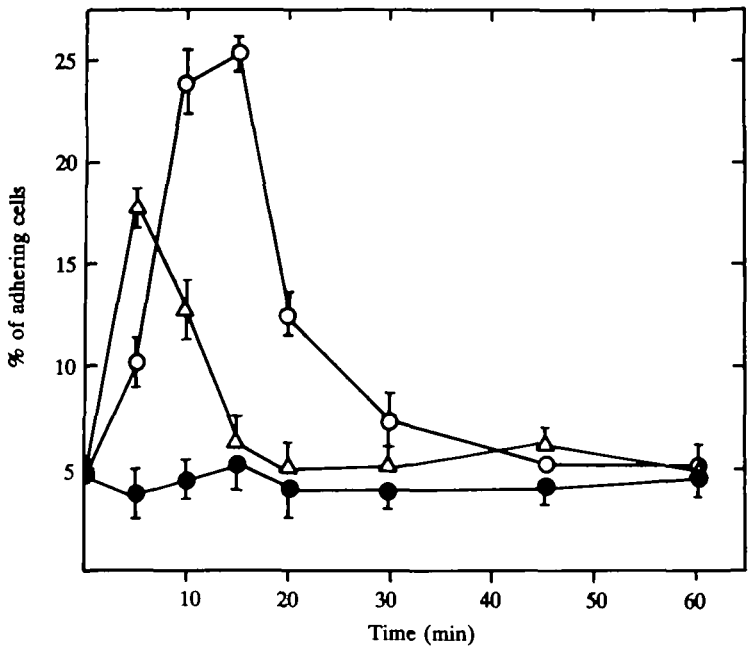

Fig. 4. Time-course of fMet-Leu-Phe and PMA-induced adherence. The cells were maintained in suspension by gentle stirring. At time zero, one group was treated with fMet-Leu-Phe (10^{-6}M) (△——△) and the second group was treated with PMA (250 ng/ml) (○——○); control (●——●). At the times shown, cells from each group were added to duplicate columns and the adherence assay was carried out as described in Materials and Methods. Shown are the results of one experiment ($\bar{y} \pm$ S.E.M.). The experiment was run twice.

specificity of the tumour cell formyl-peptide binding sites shows marked similarities to the formyl-peptide receptors on the neutrophil of the rat (Marasco *et al.* 1983) (the species from which this tumour cell was derived) and other species (Williams, Snyderman, Pike & Lefkowitz, 1977; Aswanikumar *et al.* 1977; Snyderman & Pike, 1980; Snyderman & Fudman, 1980; Spilberg, Mehta, Daughaday & Simchowitz, 1981; Weinberg, Muscato & Niedel, 1981; Niedel, Wilkinson & Cautrecases, 1979*b*). In particular, removal of the formyl group, as in Met-Leu-Phe, decreased binding 100-fold as compared to fMet-Leu-Phe. Thus as demonstrated across species lines and with different cell types, these data suggest that there is some unique property of the formyl group responsible for its dramatic effect (Freer *et al.* 1980; Marasco *et al.* 1983). Also in position 3, the phenylalanine residue has been shown to increase binding and biological activity dramatically over other non-polar and polar amino acids (Showell *et al.* 1976). With the tumour cells, we find the same results. A greater than 100-fold decrease in binding activity is seen when one replaces the non-polar amino acid phenylalanine with the negatively charged polar amino acid glutamic acid. This suggests that the hydrophobicity and/or aromaticity of phenylalanine is responsible for its profound effects. Thus, these data are

Table 1. *Comparison of the ability of various synthetic oligopeptides to displace specific fMet-Leu-[^{3}H]Phe binding and induce nylon fibre adherence*

Peptide	Inhibition of fMet-Leu-[^{3}H]Phe binding (ID_{50})*	Nylon-wool fibre adherence (ED_{50})†
Agonists		
fMet-Leu-Phe-Phe	$3{\cdot}0\times10^{-8}$	$3{\cdot}2\times10^{-9}$
fMet-Leu-Phe	$4{\cdot}0\times10^{-8}$	$6{\cdot}1\times10^{-9}$
fMet-Met-Met	$4{\cdot}1\times10^{-6}$	NA‡
fMet-Phe	$9{\cdot}0\times10^{-6}$	NA
fMet-Phe-Met	$4{\cdot}1\times10^{-5}$	NA
Met-Leu-Phe	$5{\cdot}4\times10^{-5}$	NA
fMet-Leu-Glu	$6{\cdot}8\times10^{-5}$	NA
fMet	$>>1\times10^{-4}$	NA
D,L-Met	$>>1\times10^{-4}$	NA
Antagonists		
t-boc-Phe-Leu-Phe-Leu-Phe	$4{\cdot}0\times10^{-6}$	NA
CBZ-Phe-Met	$9{\cdot}8\times10^{-5}$	NA

*ID_{50} is the molar concentration of peptide required to displace 50% of the maximum displaceable specific fMet-Leu-[^{3}H]Phe binding, which equalled approximately 45% of the total fMet-Leu-[^{3}H]Phe bound. Values are means of triplicate determinations varying <10%.

†ED_{50} is the molar concentration of peptide that gave 50% of the maximal percentage of adherence cells, which equalled $13{\cdot}1 \pm 2{\cdot}3$ and $17{\cdot}6 \pm 2{\cdot}5$ (mean ±S.E.M.) for fMet-Leu-Phe-Phe and fMet-Leu-Phe, respectively. Each value consists of the average of three independent experiments. Duplicate columns were used in each experiment. Statistical significance levels between values obtained in the presence of the peptide and control values ($3{\cdot}3 \pm 1{\cdot}1$) (mean ±S.E.M.) for that particular group of experiments were determined using Student's *t*-test and found to be highly significant.

‡NA, not active. These peptides failed to elicit a biological response over a dose range of 10^{-12}M to 10^{-6}M. Six serial 10-fold dilutions of each peptide were examined.

compatible with the specificity of the models for the rabbit (Freer *et al.* 1980, 1982; Marasco *et al.* 1982*c*) and rat (Marasco *et al.* 1983) neutrophil formyl-peptide receptors.

Correlation of binding inhibition and biological response

Since the biological activity of the peptides is known to be proportional to their binding affinity the adherence response was also used to assay the binding site specificity. Table 1 summarizes these results. Of the 10 oligopeptides tested, only the two peptides indicated in Table 1 induced a biological response in this assay with an order of potency: fMet-Leu-Phe-Phe > fMet-Leu-Phe. These two peptides were also the most active in their ability to inhibit fMet-Leu-[^{3}H]Phe binding (Table 1). In agreement with the leucocyte data (Freer *et al.* 1980; Marasco *et al.* 1983), fMet-Leu-Phe-Phe was a more potent agent than fMet-Leu-Phe, and both of these peptides induced a biological response in the same concentration range as seen in several species of leucocytes. In addition, since the ED_{50} values (see legend to Table 1) for nylon-wool fibre adherence are lower than the ID_{50} values for inhibition of fMet-Leu-[^{3}H]Phe binding, it appears that occupancy of only a fraction of the receptors is required to induce a maximum adherence response. Similar results have

Table 2. *Inhibition of formyl-peptide-induced adherence of Walker cells by t-boc-Phe-Leu-Phe-Leu-Phe*

Treatment*	% Adherence (mean ±S.E.M.)	*P*-value
None	13 ± 2	—
10^{-6}M-t-boc-Phe-Leu-Phe-Leu-Phe	10 ± 1	NS
10^{-6}M-fMet-Leu-Phe (fMLP)	23 ± 3	<0·02
10^{-6}M-fMLP+10^{-6}M-t-boc-Phe-Leu-Phe-Leu-Phe	11 ± 1	NS
None	6 ± 1	—
500 ng PMA	26 ± 5	<0·01
500 ng PMA+10^{-6}M-t-boc-Phe-Leu-Phe-Leu-Phe	23 ± 5	<0·01
50 ng PMA	18 ± 3	<0·01
50 ng PMA+10^{-6}M-t-boc-Phe-Leu-Phe-Leu-Phe	14 ± 3	<0·05

*Each value is the average of six replicate values (duplicate columns in three separate experiments; $N = 6$). For each agent examined, the test groups were run in parallel with the controls and the averages of each test group were compared with the average of the respective controls. The significance levels were determined using Student's *t*-test.

been reported with leucocytes, for chemotaxis and lysosomal enzyme secretion (Becker, 1979; Freer *et al.* 1980; Marasco *et al.* 1983). However, in contrast, several peptides that are potent agonists of the leucocyte formyl-peptide receptors, had no effect in the adherence assay at the highest concentration tested, i.e. 1 μM. These peptides include D,L-methionine, Met-Leu-Phe, fMet-Leu, fMet-Met-Phe, fMet-Phe and fMet-Met-Met. These results suggest that the efficacy of these non-active formyl-peptides with regard to their ability to induce an adherence response after binding to the Walker cell formyl-peptide receptors may be different from their relative efficacies, seen with leucocytes for several receptor-mediated biological responses.

Effect of competitive antagonists of the formyl-peptide receptor on nylon fibre adherence of Walker cells

There are now available several competitive antagonists of the leucocyte formyl-peptide receptor that are structurally related to the formylmethionyl peptides (Freer *et al.* 1980; Marasco *et al.* 1983; O'Flaherty *et al.* 1978*b*). These peptides specifically bind to the receptor site, competing with the agonist peptides for the site, but have no other detectable biological effect. We have investigated the inhibition of the Walker cell adherence response induced by fMet-Leu-Phe by two of these antagonists (i.e. CBZ-Phe-Met and t-boc-Phe-Leu-Phe-Leu-Phe). Table 2 summarizes our data. t-boc-Phe-Leu-Phe-Leu-Phe (10^{-6}M) completely abolished the adherence response induced by (10^{-6}M) fMet-Leu-Phe, while alone it had no effect on adherence. This inhibition shows specificity for fMet-Leu-Phe since no inhibitory effect was seen on the adherence response caused by the tumour-promoting agent, PMA. In contrast, the weaker antagonist CBZ-Phe-Met had no inhibitory effect on adherence induced by either fMet-Leu-Phe(10^{-6}M) or PMA(500 ng/ml) at the

highest concentration tested, i.e. 10^{-6}M (data not shown). Thus, these data offer further support for the hypothesis that the biological effects caused by fMet-Leu-Phe result from the binding of the formyl-peptide to a specific cell surface receptor.

DISCUSSION

The interaction of the chemotactic formylated oligopeptide fMet-Leu-Phe with a cell surface receptor on the leucocyte is known to initiate a multitude of biological responses in leucocytes. These responses include chemotaxis, cell swelling, increased foreign-surface adherence, cell-to-cell aggregation and an increased membrane permeability to Ca^{2+} (Becker, 1979; Becker *et al.* 1981). These events all occur as a result of and distal to receptor–ligand interaction. In previous studies, we have shown that the Walker 256 carcinosarcoma cells demonstrate similar biological response when stimulated with fMet-Leu-Phe (Wass *et al.* 1981; Varani *et al.* 1981; Lam *et al.* 1981; Grimstad, Sperniak, Fantone & Varani, 1982; Fantone *et al.* 1983*a*). While the similarities between the biological responses of Walker cells and leucocytes suggests that Walker cells also have specific binding sites for fMet-Leu-Phe on their surface, this has not yet been proven.

In this paper, we present evidence that the primary interaction of fMet-Leu-Phe with Walker cells is through the binding to a cell surface receptor with similar, though not identical, specificity to the formyl-peptide receptor found on leucocytes. This conclusion is based on the following evidence: (1) there is specificity in the relation of structure to activity; (2) there is saturability of fMet-Leu-[^{3}H]Phe binding to Walker cells; (3) there is reversibility of fMet-Leu-[^{3}H]Phe binding to the cell; (4) the kinetics of fMet-Leu-[^{3}H]Phe binding are in accord with the kinetics of several biological responses induced by fMet-Leu-Phe, i.e. cell swelling (Wass *et al.* 1981), increased foreign surface adherence (Varani *et al.* 1981) and mobilization of intracellular calcium (unpublished observation); (5) the biological activity of these peptides is proportional to their binding; and (6) the competitive antagonist of the formyl-peptide receptor, t-boc-Phe-Leu-Phe-Leu-Phe, completely blocks the adherence response induced by fMet-Leu-Phe but has no effect on the adherence induced by the structurally unrelated tumour promotor, PMA.

It is evident from the adherence data (Tables 1, 2 and Fig. 4) that only a small percentage (approx. 15%) of the total cells treated respond in this assay. Thus, Walker tumour cells may not be homogeneous in their ability to bind the peptide. A similar observation was made by Niedel, Kahane, Lachman & Cuatrecasas (1980) working with the HL-60 human promyelocytic leukaemia cell line. They were able to show a correlation between subtle differences in degree of differentiation with differences in ability to bind the rhodamine-tagged *N*-formyl-peptide. This suggests a possible source of error in the estimation of the number of receptor sites per cell of our radioassay binding data. If, in fact, Walker cells are not homogeneous in their ability to bind the peptide, our data would underestimate the number of receptors on the responsive cells. In fact, if the total number of sites per cell is corrected for the percentage of cells responding in the bioassay (adherence), then the number of

receptor sites per cell would more closely approximate the number of receptors found on leucocytes of several species (Snyderman & Fudman, 1980; Spilberg *et al.* 1981; Weinberg *et al.* 1981; Marasco *et al.* 1982*b*). Future work using flow cytometry techniques will allow us to address this possibility more definitively.

The findings reported here are important from at least two standpoints. First, previous studies from our laboratory and from other laboratories have shown that several factors, which can elicit stimulus-coupled responses in tumour cells *in vitro*, also influence the localization of circulating tumour cells and the subsequent formation of metastases by these cells (Hayashi, Yoshida, Ozaki & Ushijima, 1970; Ozaki, Yoshida, Ushijima & Hayashi, 1971; Lam *et al.* 1981; Orr *et al.* 1981). In the light of this, a considerable effort has been made to delineate the biological basis of the stimulus-coupled responses in metastatic tumour cells. It is hoped that once we understand how tumour cell responses to these factors influence their behaviour *in vivo*, it will be possible to develop rational approaches to interfere with them and thereby, perhaps, interfere with the metastatic process. Recent studies have identified a number of similarities between the stimulus-coupled responses in tumour cells and the better-described responses in leucocytes. In addition to similarities in functional responses, similarities in cation requirements (Grimstad *et al.* 1982) and involvement of arachidonic acid metabolites (Fantone *et al.* 1983*a*; Varani, 1982, 1984) have also recently been demonstrated. The observation described here (i.e., that the tumour cell response to at least one factor follows from the binding of the ligand to specific binding sites on the cell's surface) considerably advances our understanding of stimulus-coupled responses in tumour cells.

A second important aspect of this work is the demonstration and characterization of formyl-peptide binding sites on cells not derived from the myeloid series. A recent study by Hoover *et al.* (1980) identified saturable binding sites for NH_2-formyl-peptides on endothelial cells but binding characteristics (i.e. structure–function relationships, etc.) were not analysed. This paper presents the first characterization of a cell in this regard. In addition to the well-described effects on neutrophils and macrophages, recent studies have shown that the formyl-peptides can induce spasmogenic activity in guinea-pig ileum in a receptor-mediated fashion (Marasco *et al.* 1982*a*). However, the cellular distribution of the receptor remains unknown. Other studies have shown that fMet-Leu-Phe causes chemokinesis (non-direction locomotion) of human non-T lymphocytes obtained from peripheral blood (El-Naggar, Van Epps & Williams, 1980). High concentrations of formyl-peptides have also been shown to induce selective biological responses in basophils (Hook, Schiffman, Aswanikumar & Siraganian, 1976) and in spermatozoa (Vijayaserathy, Shivaji, Iqbal & Balaram, 1980; Iqbal, Shivaji, Vijayasarathy & Balaram, 1980). However, whether the responses in these cells are receptor-mediated has not been demonstrated. In addition, previous studies failed to demonstrate specific binding of formylnorleucyl-leucyl-[^{3}H]phenylalanine to human platelets, rat erythrocytes, rat brain membranes (Aswanikumar *et al.* 1977), or human and guinea-pig lymphocytes (Aswanikumar *et al.* 1977; Snyderman & Fudman, 1980). While the biological significance of a receptor for bacterial formyl-peptides on Walker carcinosarcoma

cells is not evident since these cells have no function in host defence as do the acute inflammatory cells, our data allow us to predict that the formyl-peptide receptor may be more common than we have anticipated and may exist on many seemingly unrelated cells. Our data further suggest that the formyl-peptide receptors may serve as a common mechanism by which cells and organisms locate and respond to bacteria (Marasco *et al.* 1984*b*).

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