Original Contributions

Contractile Effect of the Chemotactic Factors f-Met-Leu-Phe and C5a on the Human Isolated Umbilical Artery

Role of Cyclooxygenase Products and Tissue Macrophages

François Marceau, Denis deBlois, Claude Laplante, Eric Petitclerc, Guy Pelletier,
John H. Grose, and Tony E. Hugli

Factors that are chemotactic for phagocytic leukocytes are known to elicit important acute circulatory changes, and the role of circulating leukocytes in these models is controversial. To evaluate the role of the blood vessel wall in the absence of circulating cells, spiral strips of human umbilical artery were exposed in vitro to the chemotactic peptides f-Met-Leu-Phe (1–100 nM) or C5a (2.5–25 nM). Contractile responses were observed for both peptides. Certain agonist analogues and a selective antagonist of the chemotactic action of f-Met-Leu-Phe behaved correspondingly as agonists and antagonist of the contractile effect on umbilical artery. The anaphylatoxin C3a also exerted a contractile effect on the tissue (25 nM and above), but this effect was highly tachyphylactic. Inhibitory drugs were used to examine the contributions of secondary mediators in eliciting the effects of C5a and f-Met-Leu-Phe. The contractile effect of both peptides was massively inhibited either by indomethacin or the thromboxane A2/prostaglandin H2 antagonist SQ 29548. Dazmegrel, a thromboxane A2 synthetase inhibitor, had partial inhibitory effects on contractions induced by either peptide. The contractile effect of C3a was prevented by indomethacin pretreatment. Vascular strips did not release measurable histamine in the bathing fluid after challenge with C5a or f-Met-Leu-Phe. The tissue apparently contains neither histamine nor mast cells. Autoradiography of 125I-labeled C5a or f-Met-Leu-Phe analogue showed specific binding of the peptides to cells dispersed in the vessel wall, but more frequently at the periphery. Cells stained positively for α-naphthyl acetate esterase showed a similar distribution. Pure cultures of smooth muscle cells derived from the umbilical artery failed to release prostanoids when exposed to f-Met-Leu-Phe or C5a, whereas fresh strips of this artery released more thromboxane B2 than the baseline in response to these peptides. We conclude that macrophagelike cells, present in the vessel wall, are the likely target cells for the chemotactic peptides. These cells trigger a contractile effect of the smooth muscle by generating cyclooxygenase products. (Circulation Research 1990;67:1059–1070)

The formylated peptide f-Met-Leu-Phe and the complement-derived peptide C5a are two well-characterized factors chemotactic for human leukocytes. C5a is a member of basic homologue peptides, the anaphylotoxins, that are pharmacologically active by-products of the complement proteolytic cascade. The other anaphylotoxins are C3a and C4a; all the anaphylotoxins are histamine releasers, but only C5a is a chemotactic agent. The formylated peptide f-Met-Leu-Phe is a synthetic analogue of the NH2-terminal sequence of bacterial and mitochondrial proteins, whose synthesis is initiated with f-Met. Presumably, leukocytes have evolved the capacity to recognize directly such proteins, which should not be found in the extracellular compartment of vertebrates. Therefore, the presence of chemotactic peptides in tissues is typical of a very

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wide array of infectious, immunopathological, and traumatic processes.

Chemotactic peptides stimulate several functions besides expressing chemotactic activities. They promote adherence, stimulate production of oxygen radicals, enhance arachidonate metabolism, and induce secretion of granular materials in phagocytic leukocytes from several species, including humans. Well-defined receptors for these factors have been characterized on mononuclear cells and neutrophils. Neutrophil receptors for f-Met-Leu-Phe and for C5a are distinct, based on binding evidence, and both types are internalized and later reexpressed after occupancy.

It is perhaps not fully appreciated that chemotactic peptides can affect the circulation profoundly. In a variety of in vivo or in vitro models, anaphylatoxin C5a is a vasoconstrictor agent in the pulmonary, coronary, and renal circulation. A central role for circulating granulocytes has been postulated in one of these systems, the porcine coronary circulation, and also in the coronary vasospasm experienced by rabbits injected with f-Met-Leu-Phe. However, there is also solid evidence for the occurrence of C5a-induced arterial vasoconstriction in vitro in the absence of circulating cells—for instance, in perfused kidneys or in live rabbits after nitrogen mustard-induced neutropenia. Consistent with a direct vascular responsiveness to chemotactic factors, several animal vascular smooth muscle preparations maintained in leukocyte-free physiological fluids respond mechanically to C5a or to f-Met-Leu-Phe.

In the present study, we investigate the effect of f-Met-Leu-Phe and C5a on strips of a human vessel, the umbilical artery, in an effort to demonstrate the intrinsic responsiveness of vascular tissue to these agents in a human model. Specific issues addressed in this study include the identification of the vascular cell type responsible for the actions of chemotactic peptides, with reference to smooth muscle, endothelium, and leukocytes present in the connective structure of the vessel wall. It is already known that endothelial cells derived from the umbilical vein do not bind or respond to C5a and that responses to f-Met-Leu-Phe in rabbit isolated vessels were not prevented when the endothelium was removed.

Another point of interest in the analysis of chemotactic peptide cardiovascular actions is the involvement of secondarily released mediators. The role of arachidonate metabolites has been demonstrated pharmacologically in several models of C5a-induced or f-Met-Leu-Phe-induced vascular responses. However, this analysis is complicated by the fact that C5a is a histamine releaser from basophils and mast cells, and accordingly, a minor histaminic component of the responses was identified pharmacologically in some of the models cited above. In this study, we characterize a vascular response to C5a totally independent of mast cell mediators.

**Materials and Methods**

**Drugs**

The following drugs and compounds were purchased from Sigma Chemical Co., St. Louis: f-Met-Leu-Phe, Met-Leu-Phe, N-t-BOC-Phe-d-Leu-Phe (BPPLP), human recombinant C5a from Escherichia coli, histamine dihydrochloride, indomethacin, cycloheximide, and PAF-acether. The f-Met-Leu-Phe analogue used for iodination, f-Nle-Leu-Phe-Nle-Tyr-Lys, was purchased from Bachem, Inc., Torrance, Calif. Dazmegrel, an inhibitor of thromboxane (TX) synthetase, was a gift from Pfizer Inc., Groton, Conn. SQ 29548, an antagonist of receptors for TXA2 and prostaglandin endoperoxides, was a gift of Squibb, Princeton, N.J. Synthetic leukotrienes were donated by Merck Frosst, Montreal. A 5-lipoxygenase inhibitor, AA-861, was obtained from Takeda Chemical Industries, Osaka, Japan. Human anaphylatoxin C3a, used in comparative experiments, was chromatographically purified from human activated plasma as described previously.

f-Met-Leu-Phe, indomethacin, and dazmegrel were dissolved in 0.1 M Na2CO3; C5a was dissolved in saline containing 0.25% bovine serum albumin; and SQ 29548 was dissolved in ethanol and further diluted in 0.1 M Na2CO3. Other drugs were dissolved in 0.9% saline.

**Isolated Blood Vessels**

Human umbilical cords stored at 4°C were obtained within 24 hours from normal deliveries. Segments of umbilical arteries were carefully dissected from the cords, and a metal rod was inserted in the lumen. Excess connective tissue was excised, and helical strips were cut. In a previous study, spiral and longitudinal strips have been shown to respond in a similar manner to several agents. Strips of umbilical arteries (10–15 mm long and 2–3 mm wide) were tied at both ends and suspended in 5-ml organ baths containing oxygenated (95% O2 and 5% CO2) and warmed (37°C) Krebs’ solution. A baseline tension of 1 g was applied to the tissues. Tension changes were recorded using isometric transducers (model 52-9545, Harvard Bioscience, South Natick, Mass.) and a Harvard chart recorder.

**Protocols**

Tissues were equilibrated for 1 hour, and the baseline tension was adjusted periodically to 1 g during this period. The effect of each agent then was assessed by injecting a small volume (10–50 μl) directly in the bathing fluid. Agents were sequentially applied to four times at 1.5-hour intervals with extensive washings between each test to evaluate the presence and extent of tachyphylaxis under these standardized conditions. The effects were recorded as contractions, and responses from each agent were expressed as a percentage of an internal standard. The maximal contraction induced by histamine...
(10 μM) was measured at the end of the experiment to serve as the 100% contraction standard.

To study secondary mediator involvement in response to f-Met-Leu-Phe and C5α, several drugs were introduced to the organ baths. Development of responses to agents in the presence of drugs applied throughout the in vitro incubation period included two successive applications of either chemotactic peptide. A recording of the standard histamine-induced contraction was performed at the end of the experiment. None of the drug treatments appeared to inhibit histamine-induced contraction, except for antihistamine drugs, thus validating the use of this standard response to express the relative results. Drug concentrations were chosen on the basis of their proven effectiveness in various in vitro systems; notably, cycloheximide inhibits 35S-methionine incorporation into vascular strips by 84% at 71 μM; AA-861 inhibited completely leukotriene formation in strips of guinea pig trachea at 10 μM; the half-maximal inhibitory concentration of dazmegrel against purified human blood platelet TXA2 synthetase is 18 nM, but increases up to 30 μM in whole cells.

Although the endothelium could be damaged to a variable extent after storing and processing of the tissue, a series of experiments were designed to remove the endothelial cell layer of these tissues. These studies may exclude the participation of endothelium-derived factors in the chemotactic peptide effects. Filter paper was smeared repeatedly on the intimal surface of the artery strips to remove the endothelium before the tissues were mounted in the baths.

Assay for Histamine Release

Histamine concentrations in the Krebs’ solution bathing the vascular strips were determined by a radioenzymoassay based on histamine N-methyl transferase. Tryptic S-adenosyl-methylmethionine (60–85 Ci/mmol, Amersham, Oakville, Canada) was the methyl group donor. Standard curves for histamine were established in the presence of Krebs’ solution; sensitivity was usually 200 pg/ml. Histamine release during two consecutive 5-minute periods was determined; the first was a control period, and then a stimulant (e.g., C5α or f-Met-Leu-Phe) was applied at the beginning of the second period. Tissues used for this assay were exposed to a peptide only one time to avoid tachyphylaxis of histamine release. Aliquots (250 μl) of the bath fluid were taken immediately after tissues were washed with fresh Krebs’, 5 minutes later just before the stimulant was applied, and then 5 minutes after stimulation. Release during each period was estimated by subtracting the histamine concentration at the beginning of one period from the concentration at the end of that period. Tissues included in this study were isolated human umbilical artery and guinea pig portal vein and pulmonary artery. The guinea pig vessels were prepared as described previously18 and incubated under the same conditions as the umbilical artery. Vascular strips of human and guinea pig origin also were homogenized between glass and Teflon in 0.01N HCl to measure tissue histamine. In this case, tissue remnants were removed by centrifugation, and the homogenates were neutralized before the radioenzymoassay was performed.

Assay for Prostanoid Release From Cultured Smooth Muscle Cells or Fresh Strips of Umbilical Artery

To characterize prostanoid release from arterial smooth muscle cells in response to chemotactic peptides, cultured muscle cells were obtained in a pure form from the human umbilical artery. The vessels (approximately 40 cm) were excised from the umbilical cord and cleaned from any adherent tissue. The lumen was washed extensively to remove blood clots. Tissues were finely cut with scissors and digested at 37°C for 4 hours in medium 199 containing bacterial collagenase (type II, 3 mg/ml), elastase (type III, 1 mg/ml), and hyaluronidase (type IV-S, 0.1 mg/ml) (all enzymes from Sigma). Dispersed cells were filtered through six layers of sterile gauze and seeded at a density of 10^5/ml in 12-well plates (Linbro, McLean, Va.) precoated with gelatin (0.2%) and containing medium 199 plus 10% fetal bovine serum and antibiotics. Cells were passaged at confluency with a brief trypsin-EDTA treatment. A narrow, pointed morphology was exhibited by these cells (phase-contrast microscopy).

For the assay of prostanoid release, cells were used at passage 1 or 2. They were seeded at a density of 10^5 per well (diameter, 24 mm) and allowed to adhere for 48 hours in the culture medium described above. The cells then were washed three times with Earle’s balanced salt solution (EBSS) (Gibco Laboratories, Grand Island, N.Y.) and equilibrated at 37°C in the presence of 5% CO2. The solution in wells then was replaced by 1.5 ml EBSS containing a dissolved test substance or no added substance (controls). Stimuli included a concentration of C5α or f-Met-Leu-Phe highly active on umbilical artery strips, as well as histamine, a known stimulant of prostaglandin release from human umbilical vein smooth muscle.33 Ionomycin (Calbiochem Corp., La Jolla, Calif.) also was used as a receptor-independent stimulus for prostaglandin release. After 15 minutes of equilibration, aliquots of the supernatant were removed and frozen and later applied to specific radioimmunoassays for TXB2, and 6-ketoprostaglandin F1α (6-keto-PGF1α).34–36

After the stimulation with agonists, the cells were fixed in p-formaldehyde for 30 minutes and stained for nonspecific esterase to evaluate the possible contamination with macrophagelike cells. No cells stained for α-naphthyl acetate esterase were observed in these passaged cultures. More than 90% of the cells expressed smooth muscle-specific actin by indirect immunofluorescence (primary serum was a polyclonal antibody raised against chicken gizzard actin, Biomedical Technologies, Stoughton, Mass.). For comparison, strips of human umbilical artery were prepared as described above. Each tissue was
incubated for 5 minutes in a separate test tube containing warmed EBSS and was rinsed delicately three times with fresh EBSS. The same stimuli applied to the cultured cells were applied in 1.5-ml volumes of EBSS to the strips. The supernatants also were collected 15 minutes later for radioimmunological determination of prostanooids. The wet weight of the tissues was obtained after the procedure to normalize the prostaglandin output per milligram of tissue.

Radioiodination of Chemotactic Peptides

Iodinated f-Nle-Leu-Phe-Nle-Tyr-Lys has been used previously as a ligand for f-Met-Leu-Phe receptors on human neutrophils. The peptide f-Nle-Leu-Phe-Nle-Tyr-Lys was dissolved, with warming in dry dimethylformamide/triethylamine (99:1), to a final concentration of 1 mg/ml. The peptide was freshly labeled with iodine-125 using a mild chloramine-T reaction and purified by reversed phase high-performance liquid chromatography (Radial Pack C18 column, 8 mm x 10 cm, Waters Chromatography Div., Millipore Corp., Milford, Mass.). The mobile phase was a linear gradient of 100% phosphoric acid/triethylamine buffer at pH 3, ending with 60% acetonitrile in 40% of the buffer. The first major peak eluting after free iodine was assumed to be the monoiiodinated peptide possessing the maximal theoretical specific activity (2,200 Ci/mmol).

Human C5a, isolated and purified to homogeneity, was radiolabeled with iodine-125 by using a solid-phase lactoperoxidase method as previously described. The preparation used had a specific activity of 60 Ci/mmol.

Histology and Autoradiography

Frozen sections (10 μm thick) of the umbilical artery were used to localize and identify target cells for chemotactic peptides. Segments of umbilical arteries were removed from cords, embedded in Tissue Tek optimum cutting temperature compound (Miles Scientific, Naperville, Ill.), and frozen in liquid nitrogen. Sections (10 μM) were cut in a cryostat at −20°C, dried at 4°C overnight, and stored at −20°C. In some experiments, macrophagelike cells were stained for “nonspecific” esterase according to Yam et al using the α-naphthyl acetate esterase kit from Sigma Diagnostics. Indirect immunofluorescence also was used to locate markers for other cell types present in tissue sections: factor VIII–related antigen (von Willebrand factor) for endothelial cells and heavy-chain myosin for smooth muscle. The commercial primary antisera were raised in sheep against human von Willebrand factor (Atlantic Antibodies, Scarborough, Me.) and in rabbit against bovine uterine myosin (Biomedical Technologies Inc., Stoughton, Mass.), respectively; appropriate fluorescein isothiocyanate–labeled second antisera were used to reveal the staining.

For autoradiographic localization of 125I-f-Nle-Leu-Phe-Nle-Tyr-Lys and 125I-C5a receptor binding, slides were stored frozen as described above, then warmed to room temperature and incubated for 2 hours at 4°C in 77 mM sodium phosphate buffer (pH 7.4) containing 10% sucrose, 1 mg/ml of either protamine (for the f-Met-Leu-Phe analogue) or bovine serum albumin (for C5a), 0.3 mg/ml iodotyrosine, and 1 nM 125I-f-Nle-Leu-Phe-Nle-Tyr-Lys or 0.7 nM 125I-C5a. Controls for nonspecific binding were incubated in the same medium with 1 μM of either unlabeled f-Met-Leu-Phe or C5a, respectively.

After incubation, tissue sections were washed twice for 15 seconds at 4°C with agitation in 77 mM phosphate buffer containing 10% sucrose, then dried rapidly under cold dry air. The slides were coated with NTB-2 emulsion (Eastman Kodak Co., Rochester, N.Y.). After 48 hours of exposure, the slides were developed.

Statistical Analysis

Numerical results are mean±SEM. Statistical significance of the results was evaluated with an analysis of variance followed by the Dunnett’s test when several treatments were compared with a single control group.

Results

Contractile Effect of f-Met-Leu-Phe and Related Peptides

The chemotactic peptide f-Met-Leu-Phe (100 nM) elicited a contractile effect on resting human umbilical artery strips after at least four successive applications at 1.5-hour intervals (Figure 1A). A latency period of 30–40 seconds was always observed after application of f-Met-Leu-Phe. The contractile response to f-Met-Leu-Phe reached a maximum in 3–4 minutes. A brief contraction was seen in response to washing many tissues independent of the stimulating agent. The response to this peptide increased slightly from the first to the second exposure and remained stable for the third application. Minor tachyphylaxis was evident at the fourth exposure (Figures 1A and 2a).

A concentration–effect study showed that f-Met-Leu-Phe elicited contractions of the arteries at and above 0.1 nM in a concentration-dependent manner (Figure 2a). Variations in the response also were seen as a function of repeated applications of the peptide at low concentrations. The effect of the first application was usually inferior to that of subsequent challenges.

Both structure–activity and pharmacological antagonism data were obtained for f-Met-Leu-Phe (Figure 3). The contractile response to f-Met-Leu-Phe (100 nM) was practically abolished in the presence of a selective antagonist of formylated peptides on leukocytes, BPLLP (10 μM) (Figure 3A). This antagonist had no effect on the contractile response to histamine (10 μM). Umbilical arteries were not responsive to the nonformylated peptide Met-Leu-Phe (100 nM) (Figure 3B), f-Nle-Leu-Phe-Nle-Tyr-Lys (100 nM) was approximately equipotent with f-Met-Leu-Phe (100 nM) for contracting umbilical arteries, thus validating the use of this analogue, in the iodinated form, as a ligand in autoradiography.
Contractile Effect of C5a

The chemotactic peptide C5a elicited a contractile effect on resting isolated human umbilical arteries similar to f-Met-Leu-Phe. Figure 1B shows the effect of three successive challenges at 1.5-hour intervals. The latency of 30–40 seconds also was observed between applications of C5a and the contraction. The contractile response of C5a reached a plateau in 3–4 minutes. The response amplitude to this peptide increased from the first to the third exposure (Figures 1B and 2b) and appeared to be concentration related (2.5 and 25 nM concentrations were tested, Figure 2b).

FIGURE 1. Contractile effect of chemotactic peptides on isolated human umbilical arteries. Panel A: Effect of f-Met-Leu-Phe (100 nM) applied consecutively four times at 90-minute intervals. Panel B: Effect of recombinant C5a (25 nM) applied consecutively three times at 90-minute intervals. In each tissue, the maximal contraction induced by histamine (10 μM) was recorded as internal standard. Abscissa scale: time. Closed triangles refer to application of agents; open triangles refer to first washout of stimulants.

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FIGURE 2. Concentration–effect relation for chemotactic peptide–induced (panels a and b) or C3a-induced (panel c) contractions of isolated human umbilical artery. Curves were constructed by assigning tissues to one concentration of a single peptide, which was applied two to four times at 90-minute intervals. Results are the means of six to 29 determinations and are expressed as a percentage of the maximal histamine-induced response established in each tissue; vertical bars indicate SEM.
Effect of Inhibitory Drugs on the f-Met-Leu-Phe–Induced and C5a–Induced Contractions

The mechanism of C5a-induced and f-Met-Leu-Phe–induced vascular contractions was explored (Table 1). The release of secondary mediators by f-Met-Leu-Phe and C5a from tissues, and especially from leukocytes, is known to occur, and this has guided our pharmacological approach.

The contractile effect of either f-Met-Leu-Phe or C5a was inhibited to a considerable extent (85–90%) in the presence of the cyclooxygenase inhibitor, indomethacin (2.8 μM). The inhibitory effect of this drug was observed in two consecutive exposures of umbilical arteries to f-Met-Leu-Phe. The thromboxane receptor antagonist, SQ 29548 (100 nM), exhibited an extensive inhibitory effect similar to indomethacin. The thromboxane synthetase inhibitor, dazmegrel (40 μM), reduced partially the contractile response to f-Met-Leu-Phe and to C5a, but this inhibition reached statistical significance only for second exposures of either peptide. A lower concentration (1.9 μM) of dazmegrel reduced the effect of f-Met-Leu-Phe by a similar fraction, suggesting that this fraction represents the maximal inhibitory effect of the drug. The 5-lipoxygenase inhibitor, AA-861,

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**Table 1. Effect of Drugs on Chemotactic Peptide–Induced Contractions of Umbilical Artery Strips**

<table>
<thead>
<tr>
<th>Drug (concentration) or treatment</th>
<th>Effect of f-Met-Leu-Phe (100 nM)</th>
<th>Effect of C5a (25 nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st exposure</td>
<td>2nd exposure</td>
</tr>
<tr>
<td>Control (none)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intimal damage</td>
<td>30.5±2.6 (36)</td>
<td>49.4±3.7 (36)</td>
</tr>
<tr>
<td>Indomethacin (2.8 μM)</td>
<td>31.1±3.7 (11)</td>
<td>44.9±4.5 (11)</td>
</tr>
<tr>
<td>SQ 29548 (100 nM)</td>
<td>5.0±1.9 (6)*</td>
<td>6.4±3.5 (6)*</td>
</tr>
<tr>
<td>Dazmegrel (1.9 μM)</td>
<td>18.2±2.1 (6)</td>
<td>22.8±4.9 (6)*</td>
</tr>
<tr>
<td>Dazmegrel (40 μM)</td>
<td>18.3±4.0 (6)</td>
<td>16.3±5.4 (6)*</td>
</tr>
<tr>
<td>Cycloheximide (71 μM)</td>
<td>44.5±9.4 (6)</td>
<td>62.8±9.2 (6)</td>
</tr>
<tr>
<td>AA-861 (1 μM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA-861 (10 μM)</td>
<td>22.8±5.2 (8)</td>
<td>56.4±8.5 (8)</td>
</tr>
<tr>
<td>Analysis of variance</td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

Values are mean contraction±SEM, expressed as a percentage of maximal histamine-induced contraction determined in each tissue. Different sets of tissues were used for f-Met-Leu-Phe and C5a stimulations and for each drug. Control groups included tissues from all individual cords represented in treated groups. Numbers in parentheses are number of determinations. Analysis of variance performed on columns of data showed experimental groups were not homogeneous in all cases. Dunnett’s test was used to compare each drug-treated group with its control group.

*p<0.01.

†p<0.05.
was used at high concentration (10 μM) and had no significant effect on the response of arteries to f-Met-Leu-Phe; however, it partially inhibited C5a-induced contractions, statistical significance being reached for the second exposure to the protein. At 1 μM, the drug AA-861 did not inhibit C5a-induced responses. Cycloheximide, a protein synthesis inhibitor, failed to influence the contractile effect of two consecutive applications of either f-Met-Leu-Phe or C5a.

The combination of histamine receptor antagonists, cimetidine (12 μM) and pyrilamine (5 μM), had no significant effect on either f-Met-Leu-Phe-induced or C5a-induced contractions. These results were not included in Table 1 because of the inhibitory effect of pyrilamine on the standard histamine contraction, and the interpretation is based on the average absolute contractions, expressed in grams, obtained with or without drugs (not shown).

Mechanical removal of the endothelium had no significant effect on the contractions induced by these chemotactic peptides (Table 1).

Exogenous leukotrienes B4, C4, and D4 (100 nM each) elicited relatively weak contractile effects on resting umbilical artery (9.9±5.6%, 9.5±5.4%, and 11.1±7.7% of the histamine standard response, respectively; n=6, 7, and 9, respectively). Exogenous PAF-acether (100 nM) had no effect on resting umbilical artery (in three different tissue preparations).

**Contractile Effect of C3a**

The anaphylatoxin C3a is not a chemotactic peptide, but its amino acid sequence is homologous to that of C5a; also, C3a shares with C5a the capability to activate certain leukocyte types.1 Purified human C3a contracted the isolated umbilical artery; the contraction profile was similar to that of C5a (not shown), but important differences were noted. First, the concentration range to obtain contractions was higher for C3a (25 nM and above) than for C5a (Figure 2c). Second, C3a-induced contractions were highly tachyphylactic, and no responses were recorded at the second exposure to the peptide, even 90 minutes after recording the first response (Figure 2c). C3a-des-Arg failed to induce any response in fresh tissues at 2.5 μM (n=6), in agreement with the known structure-activity relation for that peptide in several bioassays.1 When indomethacin (2.8 μM) was introduced into the bathing fluid of fresh tissues, the latter failed to contract in the presence of C3a (2.5 μM, n=4) but responded to histamine.

**Study of Histamine Release From Umbilical Arteries by Chemotactic Peptides**

Neither f-Met-Leu-Phe nor C5a challenges released detectable histamine in the bathing fluid of human umbilical arteries (Table 2). By comparison, the portal veins and pulmonary arteries isolated from guinea pigs released measurable histamine when challenged with C5a under the same conditions. The two latter vessels were included as positive controls, as the vasoconstrictor effect of C5a on these vessels has been shown pharmacologically to be partly dependent on histamine release.18 Histamine present in the human umbilical artery and guinea pig portal vein and pulmonary artery was evaluated after tissue homogenization and acid extraction (Table 2). The human tissue contained no measurable histamine, whereas the guinea pig tissues contained appreciable levels.

**Studies of Histological Sections**

The general architecture of frozen tissue sections of the human umbilical artery is shown in Figure 4. Specific cell types have been localized using special methods. Vascular smooth muscle cells and endothelium were shown by indirect immunofluorescence for heavy-chain myosin and factor VIII-related antigen, respectively. No mast cells could be detected using toluidine blue staining and various forms of tissue fixation, including Carnoy’s fixative (not shown). Acidine orange fluorescence for mast cells also was negative. Cells positive for α-naphthyl acetate esterase, interpreted as macrophagelike,40 have been localized in umbilical arteries (Figure 4). The number of these cells was highly variable in a given section. Tissue sections 10 μm thick often showed 30–50 tissue macrophages. Although these cells were observed throughout the vessel wall, their density was maximal at the periphery.

Populations of cells possessing binding sites for the iodinated analogue of f-Met-Leu-Phe or for C5a have been localized in umbilical arteries (Figure 5). The background was not interpreted as an even distribution of a low density of specific binding sites, because it was similar in sections incubated with an excess of

<table>
<thead>
<tr>
<th>Blood vessel</th>
<th>Histamine in bathing fluids</th>
<th>Stimulation with f-Met-Leu-Phe (100 nM)</th>
<th>Stimulation with C5a (25 nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average wet wt (mg)</td>
<td>Basal period</td>
<td>Stimulated period</td>
</tr>
<tr>
<td>Human umbilical artery</td>
<td>48</td>
<td>0 (8)</td>
<td>0 (8)</td>
</tr>
<tr>
<td>Guinea pig portal vein</td>
<td>43</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Guinea pig pulmonary artery</td>
<td>36</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Values show mean histamine release in bathing fluid (ng per tissue) during two consecutive 5-minute periods, a basal release period followed by a chemotactic peptide–stimulated one. Number of determinations indicated in parentheses.

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cold f-Met-Leu-Phe or C5a (1 μM). However, the cellular pattern of high-density binding sites was abolished in the presence of cold f-Met-Leu-Phe or C5a (Figure 5). These cells had a distribution similar to the esterase-positive cells determined on other sections (Figure 4).

Prostanoid Release From Umbilical Arteries: Comparison of Fresh Tissues With Cultured Smooth Muscle Cells

Strips of fresh umbilical arteries stimulated in vitro with 100 nM of f-Met-Leu-Phe or C5a failed to release 6-keto-PGF\textsubscript{1α} above the control level, but the TXB\textsubscript{2} release, determined by radioimmunoassay, was selectively stimulated (statistical significance reached for C5a) (Table 3). These prostanoids were variously elevated by a known stimulant of prostaglandin release from human umbilical smooth muscle,\textsuperscript{33} histamine (10 μM), or by a calcium ionophore, ionomycin (500 nM).

By contrast, cultured smooth muscle cells derived from the umbilical artery failed to release TXB\textsubscript{2} or 6-keto-PGF\textsubscript{1α} in response to the chemotactic peptides f-Met-Leu-Phe or C5a. The capacity to respond to histamine and ionomycin was retained in the cultured cells and expressed, although not to the level of statistical significance. Another difference between the cultured cells and the fresh tissues is the relative abundance of the released prostanoids, 6-keto-PGF\textsubscript{1α} being relatively more important in the fresh vascular tissue.
FIGURE 5. Autoradiography of $^{125}$I-labeled chemotactic peptides in transverse frozen sections of human umbilical artery. Panels A, C and E: Autoradiography with $^{125}$I-Nle-Leu-Phe-Nle-Tyr-Lys. Panels B, D, and E: Autoradiography with $^{125}$I-C5a. In panels A and B, the luminal side is up, and the full thickness of the vessel wall is illustrated ($\times 200$). Panels C and D are close-up views of cells binding labeled peptides ($\times 1,200$). Panels E and F show background in the presence of an excess of cold peptide (see "Materials and Methods") ($\times 1,200$).
Table 3. Release of Prostanoids From Cultured Arterial Smooth Muscle Cells and From Umbilical Artery Strips Under Pharmacological Stimulation In Vitro

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Release from cultured smooth muscle cells (pg/ml, n=4)</th>
<th>Release from umbilical artery strips (pg/ml/mg of fresh tissue, n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6-keto-PGF&lt;sub&gt;1α&lt;/sub&gt;</td>
<td>TXB&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>None (control)</td>
<td>13.5±13.5</td>
<td>10.8±1.8</td>
</tr>
<tr>
<td>f-Met-Leu-Phe (100 nM)</td>
<td>8.9±6.2</td>
<td>10.2±1.2</td>
</tr>
<tr>
<td>C5a (100 nM)</td>
<td>8.0±7.8</td>
<td>9.2±0.8</td>
</tr>
<tr>
<td>Histamine (10 μM)</td>
<td>28.6±3.9</td>
<td>12.9±0.9</td>
</tr>
<tr>
<td>Ionomycin (500 nM)</td>
<td>26.5±5.1</td>
<td>12.1±0.9</td>
</tr>
</tbody>
</table>

Values are mean±SEM of four determinations (from two separate cell lines) for cultured smooth muscle cells or of nine determinations (from three separate umbilical cords) for arterial strips. 6-Keto-PGF<sub>1α</sub>, 6-ketoprostaglandin F<sub>1α</sub>; TXB<sub>2</sub>, thromboxane B<sub>2</sub>.

*p<0.01, compared with control by Student’s t test.

†p<0.05, compared with control by Student’s t test.

Discussion

Both f-Met-Leu-Phe and C5a are known activators of phagocytic leukocytes. In this paper, we reported a prostanoi-mediated contractile response of human umbilical artery to f-Met-Leu-Phe and C5a and showed that the target cell type for these peptides is not smooth muscle and is likely to be the tissue macrophage.

Antagonism of the effect of f-Met-Leu-Phe by BP-LPLP, the lack of response of umbilical artery to Met-Leu-Phe and C5a shows that the target cell type for these peptides is not smooth muscle and is likely to be the tissue macrophage.

The f-Met-Leu-Phe–induced and C5a-induced vasomotor effect appeared to be mediated ultimately by arachidonate metabolites of the cyclooxygenase pathway, because an inhibitor of cyclooxygenase, indomethacin, virtually abolished the response of umbilical artery to these peptides (Tables 1 and 2). An antagonist of TXA<sub>2</sub>/prostaglandin H<sub>2</sub> that is structurally related to TXA<sub>2</sub>, SQ 29548, also reduced the effect of the chemotactic peptides, further implicating a role for prostanoids.

Cultured human endothelial cells from umbilical veins do not possess receptors for C5a<sup>21</sup> but may possess low-affinity binding sites for f-Met-Leu-
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Phe. However, the latter appear to be atypical as they do not bind f-Nle-Leu-Phe-Nle-Tyr-Lys, and occupancy by f-Met-Leu-Phe was not associated with measurable response. In the present vascular system, f-Nle-Leu-Phe-Nle-Tyr-Lys was active as a contractile agent, and its iodinated form did not reveal binding sites in the endothelium by autoradiography. Intentional intimal damage did not inhibit the effect of these chemotactic peptides. In fact, quite extensive intimal damage might have been experienced in control tissues during preparation. These facts suggest that the endothelium is not the responsive element to chemotactic peptides in our system. Pure cultured smooth muscle cells failed to release TXB₂ in response to f-Met-Leu-Phe or C₅a, in contrast with fresh strips of umbilical artery (Table 3). This suggests that the prostanoïd release by these peptides, which is necessary for the mechanical response of the muscle, is absent in the smooth muscle cell itself.

We have located a population of tissue macrophages in umbilical artery sections by histochemical studies (nonspecific esterase assay). Specific binding of labeled f-Nle-Leu-Phe-Nle-Tyr-Lys or C₅a revealed a similar cellular uptake distribution. In the absence of mast cells, it is likely that these populations coincide. These results suggest an interaction between tissue macrophages and smooth muscle cells. Macrophages dispersed in the connective structure of the vessel wall may release prostanoïds in response to chemotactic peptides that contract smooth muscle cells. Activated macrophages are a known source of TXA₂ and other prostanoïds.

The C₃a-induced contractions were highly tachyphylactic and dependent on arachidonate metabolites, essentially as described previously on guinea pig isolated vessels. The target cell type for C₃a was not determined, but functional macrophage responses to C₃ have been described previously. Therefore, it is possible that a single cell type in the tissue accounts for the response to chemotactic peptides and C₃a.

The proposed mechanism for chemotactic peptide–induced vasomotor activity may apply to a very wide array of infectious, traumatic, and immunological conditions, in reference to the release of formylated peptides by destroyed bacteria or mitochondria and to the formation of C₅a by complement activation. Complement is activated by selective immunological reactions triggered, for instance, by immune complexes. Lupus nephritis is characterized by renal immune complex deposition and complement activation. It is of interest that a TXA₂/prostaglandin H₂ receptor antagonist, BM 13,177, improves renal function and perfusion in patients with lupus nephritis, showing indirectly the effect of vasoconstrictor prostanoïds in this pathology. It is possible that a mechanism similar to the one that we have proposed here also operates in renal resistance vessels. Nonselective activation of complement also may occur in ischemic tissues, suggesting that C₅a-induced vasomotor effects may find an even larger field of application in the pathological regulation of circulation.

In reference to the formation of pharmacologically active complement fragments in vivo, it is of interest that C₅a, a nonchemotactic anaphylatoxin, is less potent and much more tachyphylactic than C₅a as a spasmogen on the umbilical artery. Therefore, C₅a appears to be more clinically relevant than C₅a as an endogenous vasoactive agent. This agrees with various animal models of in vivo complement activation, in which pulmonary hypertension and decreased cardiac output are observed⁴; purified C₅a injected intravenously produces a similar reaction in the same species.⁵,⁶

Acknowledgments

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- anaphylatoxin C3a
- anaphylatoxin C5a
- prostaglandin-dependent vasooactivint response
- umbilical artery
- chemotactic peptides
- macrophage-dependent vasoconstrictor response
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