Purification of a Monocyte Chemotactic Factor Secreted by Nonhuman Primate Vascular Cells in Culture†

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ABSTRACT: A protein chemotactic for peripheral blood monocytes (SMC-CF) of potential importance in their recruitment to the arterial intima in atherogenesis was purified from serum-free medium conditioned by cultured baboon aortic medial smooth muscle cells. The purification of SMC-CF was monitored by a filter assay using human peripheral blood mononuclear cells and was achieved by batch separation on a cation-exchange gel followed by gel permeation chromatography, ion-exchange high-performance liquid chromatography (HPLC), and reversed-phase HPLC. The overall recovery was approximately 10% of the initial activity and yielded 0.5–1 μg of SMC-CF/L of conditioned medium. On analytical sodium dodecyl sulfate–polyacrylamide gel electrophoresis, SMC-CF migrated as a monomeric protein with an apparent molecular weight of 14 500. A dose-dependent relationship was observed between SMC-CF concentration and monocyte chemotactic activity, with maximal and half-maximal biologic activity being observed at approximately 5 and 0.1 nM, respectively. Cultured baboon aortic smooth muscle cells also express the genes for both the A and B polypeptide chains of platelet-derived growth factor, which has been reported to be chemotactic for blood monocytes and neutrophils [Deuel, T. F., Senior, R. M., Huang, J. S., & Griffin, G. L. (1982) *J. Clin. Invest. 69*, 1046–1049]. Amino acid composition analyses indicate that SMC-CF is not derived either from polypeptide chain of this growth factor or from certain potentially chemotactic connective tissue proteins.

Enhanced recruitment of peripheral blood monocytes to the arterial intima is a feature of both human and experimental atherosclerosis (Duff et al., 1957; Still & O'Neal, 1962; Gerrity et al., 1979; Schaffner et al., 1980; Stary, 1980; Lewis et al., 1982; Joris et al., 1983; Schwartz et al., 1985a). Intimal monocyte-derived macrophages are thought to be important in atherogenesis since they perform several major functions related to tissue injury and repair [reviewed by Schwartz et al. (1985b, 1986)]. In addition to being precursors of many of the cholesteryl ester-rich foam cells found in intimal plaques, monocyte-derived macrophages are actively phagocytic, secrete a broad range of neutral hydrolases specific for arterial matrix and blood coagulation proteins, and produce potentially toxic reactive oxygen species. As well as these functions that are related to tissue debridement, macrophages also secrete growth-promoting factors that stimulate proliferation of the vascular smooth cells and thus facilitate tissue repair and remodeling.

The mechanisms that regulate blood monocyte recruitment to the artery wall have not been completely defined, but clearly involve the contact and adherence of the blood-borne cells to the vascular endothelium and their subsequent net directed migration into the subendothelial space. The adherence phenomenon is likely to involve changes in the surface properties of both the endothelium and the monocytes and appears to be influenced by a broad spectrum of factors, including many derived from platelets, leukocytes, and the vascular endothelium (Boxer et al., 1980; Buchanan et al., 1983; Linder et al., 1983; Hoover et al., 1984; Valente et al., 1984a; Bevilacqua et al., 1985). Following this attachment process, monocytes migrate across the endothelium into the subendothelial space. The net directed migration of these cells suggests that they recognize chemotactic gradients originating from within the artery wall.

Both the cellular and the extracellular components of the artery wall are potential sources of chemotactic activity. Several connecive tissue matrix proteins, for example, can be cleaved to release peptide fragments chemotactic for leukocytic and nonleukocytic cells (Postlethwaite & Kang, 1976; Bowersox & Sorgente, 1980; Hunninghake et al., 1981; Norris et al., 1982). A more significant source of monocyte chemotactic factors in the artery, however, may be the smooth muscle cells of the tunica media and intima. Recently, we and others demonstrated that cultured aortic medial smooth muscle cells secrete potent chemotactic activity for peripheral blood monocytes (Fowler et al., 1982; Jauchem et al., 1982; Mazzone et al., 1983; Valente et al., 1984b). These chemoattractants may significantly influence the regulation of intimal monocyte recruitment in the atherogenic process.

In previous papers, we described the partial characterization of a chemoattractant (SMC-CF)[1] that was synthesized and secreted by baboon aortic medial smooth muscle cells in culture (Fowler et al., 1982; Jauchem et al., 1982; Valente et al., 1984b). This activity was associated with a low molecular weight, basic protein(s) and was specific for blood monocytes. In this paper, we describe the complete purification to homogeneity of SMC-CF from serum-free medium conditioned

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[1] Abbreviations: SMC-CF, smooth muscle cell derived chemotactic factor; PDGF, platelet-derived growth factor; HBSS, Hank's balanced salt solution; HBSS-CD Hank's balanced salt solution, without Ca^{2+} or Mg^{2+}, with trisodium citrate (3.7 g/L) and dextrose (2.7 g/L); FBS, fetal bovine serum; $NaDodSO_4$, sodium dodecyl sulfate; RP-HPLC, reversed-phase high-performance liquid chromatography; CM, carboxymethyl; EDTA, ethylenediaminetetraacetic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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by baboon smooth muscle cells and extend our characterization studies of this protein.

Experimental Procedures

Materials. Medium 199 (M199), MEM Select-Amine kit, Hank's balanced salt solution (HBSS, L-glutamine, penicillin, streptomycin, and trypsin were obtained from Gibco Laboratories. Fetal bovine serum (FBS) was obtained from Hazelton Research Products, Inc. Bovine serum albumin, NaDodSO$_4$–polyacrylamide gel electrophoresis molecular weight protein markers, cytochrome *c* and Histopaque were obtained from Sigma Chemical Co. Poly(vinylpyrrolidone)-free polycarbonate filters (5 μm) and modified Boyden chemotactic chambers were purchased from Nucleopore Corp. Plastic tissue culture flasks were from Corning Science Products, and Spectropor dialysis tubing (molecular weight cutoff 6000–8000) was from American Scientific Products.

Smooth Muscle Cell Cultures and Conditioned Medium. Smooth muscle cells were isolated and cultured from the inner layer of the tunica media of baboon (*Papio cynocephalus*) aortas as described by Sprague et al. (1982). The cells were grown in 150-cm^2 tissue culture flasks in M199 supplemented with 5% FBS, 2 mM L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin. When confluent, the cells were subcultured at a split ratio of 1 in 4 and incubated in the above medium. Cells were not used after their 10th passage in culture.

To obtain serum-free medium, confluent cultures of cells in 150-cm^2 flasks were rinsed twice in HBSS and further incubated in 30 mL of M199 supplemented with penicillin and streptomycin only. Medium was collected and replaced with fresh serum-free medium at 2-day intervals for 6 days. Following the last collection, the remaining adherent cells were incubated in the complete serum-containing medium to stimulate growth and proliferation. The cycle of cell growth and conditioning of serum-free medium was repeated at least twice with each culture. Conditioned medium was stored at −20 °C until use.

Monocyte Chemotaxis Assay. The preparation of human peripheral blood mononuclear cells and the chemotaxis assay for SMC-CF have been described previously (Böyum, 1968;, Valente et al., 1984b). Freshly drawn human venous blood (5 volumes) was mixed with 1 volume of anticoagulant citrate–dextrose and centrifuged at 150*g* for 30 min at room temperature. The platelet-rich plasma was discarded, and the cells were resuspended to the original volume with Ca^{2+}- and Mg^{2+}-free HBSS supplemented with 2.7 g/L dextrose and 3.7 g/L trisodium citrate (HBSS-CD). The centrifugation step was repeated, the supernatant was removed, and the cells were resuspended to twice volume in HBSS-CD. Two volumes of cells were layered over 1 volume of Histopaque and centrifuged at 400*g* for 30 min. The mononuclear cells collecting at the interface were pooled, washed twice in HBSS-CD, and suspended in assay medium (M199 containing 2 mg/mL bovine serum albumin) to 3×10^6 cells/mL. Mononuclear cell preparations were >95% viable, as determined by the exclusion of trypan blue dye.

The monocyte chemotaxis assay was carried out by using poly(vinylpyrrolidone)-free polycarbonate 5-μm filters and modified (blind-well) Boyden chambers. Samples were diluted at least 2-fold in the assay medium and 0.2 mL placed in the wells below the filters. Five hundred microliters of the mononuclear cell preparations was placed above the filters, and the chambers were incubated at 37 °C in 5% CO_2/95% air for 90 min. The filters were then removed, fixed in methyl alcohol, and stained with Giemsa. Cells migrating to the underside of the filters were quantitated microscopically by determining the mean number of cells in 10 high-power (100×) fields. Previous studies have shown that the majority of cells migrating through the filters are monocytes (Valente et al., 1984b). Values obtained with control medium were subtracted from experimental data. To monitor the separation of the activity in the various chromatography steps, eluted fractions were assayed singly. For the determination of the yield of the chemotactic activity obtained in the purification procedure, solutions were assayed in duplicate and at more than one concentration. For each purification procedure, a standard curve was established by using the purified chemotactic protein obtained in that particular experiment. Samples from gel permeation chromatography were lyophilized to remove acetic acid before assay. Samples from the reversed-phase HPLC steps were tested directly at 1:1000 dilution or higher. At these concentrations there was minimal influence on the assay by the solvent compounds. A dose–response curve was obtained between mean net chemotactic activity and the concentration of the purified protein in the lower chamber. One unit of activity was defined as the amount in 0.2 mL of the assay medium that produced half-maximal net stimulation of mononuclear cell migration under the conditions of assay.

Protein Assay. The protein concentration of the smooth muscle cell conditioned medium was determined by the Bradford dye-binding technique (Bradford, 1976) using commercially available reagent (Bio-Rad Laboratories). Interference in the color reaction by medium components was compensated for by subtraction of the values obtained with unconditioned medium. The protein concentrations of the CM-Sepharose and Sephacryl S-200 eluate pools were also estimated by using this procedure. The CM-Sepharose eluate was diluted in distilled water and assayed directly. Aliquots (0.5–1.0 mL) of the Sephacryl S-200 eluate were first lyophilized and then reconstituted in 0.15 M NaCl before assay. To conserve material, the protein concentrations of the active eluates from the ion exchange and initial reversed-phase HPLC steps were not determined. The concentration of the purified protein (M_r 14 500) was determined from the amino analysis data by using L-norleucine as an internal standard.

Chromatographic Procedures. (*a*) *Ion-Exchange Chromatography.* Conditioned medium was fractionated on CM-Sepharose in 1–2-L volumes. All procedures were carried out at 4 °C. A column (25 × 150 mm) of CM-Sepharose Fast Flow (Pharmacia, Inc.) was equilibrated in 0.05 M sodium acetate adjusted to pH 5.5 with glacial acetic acid. Conditioned medium was clarified by filtration through a coarse glass fiber prefilter (Type AP, Millipore Corp.) and diluted with 2 volumes of cold glass-distilled water. Na_2EDTA was added to a final concentration of 1 mM and the pH adjusted to 5.5 with glacial acetic acid. The diluted medium was pumped through the gel at approximately 200 mL/h. The gel was then washed with 5 column volumes of equilibrating buffer, and the bound proteins eluted at 50 mL/h with 1 M NaCl and 0.05 M sodium acetate adjusted to pH 5.5 with acetic acid. Fractions containing protein were pooled, dialyzed against 6 L of 1 M acetic acid, (adjusted to pH 3.5 with ammonium hydroxide), and lyophilized.

(*b*) *Gel Permeation Chromatography.* The lyophilized material obtained from the ion-exchange step was dissolved in 2 mL of 4 M guanidine hydrochloride and centrifuged for 5 min at 12000*g*. The supernatant was applied to a 2 × 80 cm column of Sephacryl S-200 equilibrated in 1 M acetic acid, pH 3.5, and eluted in the same buffer. The column was eluted at a flow rate of 12 mL/h, and 6-mL fractions were collected.

Table I: Purification of SMC-CF from Conditioned Medium of Baboon Aortic Smooth Muscle Cells

purifictn step	vol (mL)	protein recovd (μg)	SMC-CF act. recovd (units)	rel sp act. (units/μg)	deg of purifictn (x-fold)	recovery (%)
(1) SMC-conditioned medium	1730	100 000	20 000	0.2	1	100
(2) CM-Sepharose FF	80	40 000	15 700	0.4	2	75
(3) Sephacryl S-200	35	710	12 200	17	85	61
(4) TSK SP-5 PW	10	ND[a]	6 400	ND[a]	ND[a]	32
(5) Hi-Pore C_4 (acetonitrile/TFA)	2	ND[a]	3 000	ND[a]	ND[a]	15
(6) Vydac protein/peptide C_{18} (acetonitrile/HFBA)	1	1	2 000	2900	15000	11

[a] ND = not determined.

Aliquots of the fractions were taken for the monocyte chemotaxis assay. Active fractions were pooled and lyophilized.

(*c*) *Ion-Exchange HPLC.* Ion exchange was carried out on a 75 × 7.5 mm TSK SP-5-PW column (Bio-Rad Laboratories) equilibrated in 0.05 M sodium phosphate, pH 7.0. The lyophilized fractions from the gel filtration step were dissolved in approximately 0.5 mL of the equilibrating buffer, centrifuged at 12000*g* for 5 min, and injected into the column. Bound proteins were eluted at 1 mL/min in a 0–0.5 M linear gradient of NaCl in 0.05 M sodium phosphate, pH 7.0. Elution was monitored at 220 nm, and 1 mL fractions were collected and assayed for chemotactic activity. Active fractions were pooled and stored at −20 °C.

(*d*) *Reversed-Phase HPLC.* Pooled active fractions from the previous ion-exchange chromatography step were finally separated by reversed-phase HPLC. Separations were performed on a Bio-Rad Hi-Pore C_4 column (250 × 4.6 mm) and a Vydac 5-μm C_{18} protein/peptide column (250 × 4.6). The pooled fractions were applied by multiple injection to the C_4 column equilibrated in 0.1% trifluoroacetic acid (TFA) and eluted in a linear (0–70%) gradient of acetonitrile in 0.1% TFA. Active fractions from this chromatographic step were pooled, diluted with an equal volume of 0.15% heptafluorobutyric acid (HFBA), and applied to the C_{18} column equilibrated in 27% acetonitrile in 0.15% HFBA and eluted in a linear gradient (27–55%) of acetonitrile in 0.15% HFBA. In both steps the eluate was monitored at 220 nm.

(*e*) *Metabolic Labeling of SMC Proteins with [^{35}S]-Methionine/[^{35}S]Cysteine.* Smooth muscle cells were grown in 150-cm^2 flasks in M199 supplemented with 5% FBS, 2 mM L-glutamine, penicillin, and streptomycin until confluent. Four flasks of cells were rinsed twice in HBSS and incubated for 24 h in 30 mL of MEM containing penicillin and streptomycin only. The medium was decanted and discarded and replaced with 30 mL of cysteine/methionine-free MEM (Select-Amine kit, Gibco Laboratories) containing 40 μCi/mL ^{35}S-labeled *E. coli* hydrolysate labeling reagent (ICN Radiochemicals). Incubation was continued for 48 h, after which time the labeling medium was collected and pooled and the cells were discarded. The labeled medium was pooled with 1700 mL of unlabeled smooth muscle cell conditioned serum-free medium and SMC-CF protein isolated as described above.

(*f*) *NaDodSO$_4$–Polyacrylamide Gel Electrophoresis.* NaDodSO$_4$–polyacrylamide gel electrophoresis was performed on 15% polyacrylamide (acrylamide:bis, 30:0.8) gels in a discontinuous buffer system (Laemmli, 1970). Samples and standards were dissolved in 2% NaDodSO$_4$ and 0.025 M Tris-HCl, pH 6.8 (with or without 5% β-mercaptoethanol), and boiled for 1 min before application to the gel. The gels were fixed in 50% methanol and 10% acetic acid overnight and then stained with silver (Merril et al., 1981). For autoradiography, the gels were soaked for 30 min in Amplify (Amersham Corp.), dried, and exposed to Kodak X-Omat X-ray film at −70 °C.

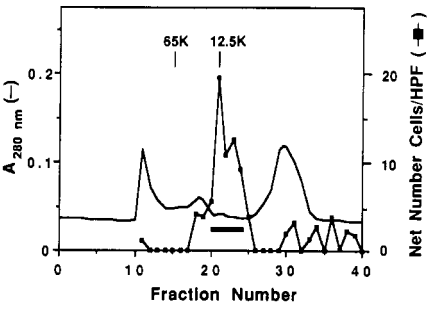

FIGURE 1: Gel permeation chromatography on a Sephacryl S-200 column (2 × 80 cm) of lyophilized protein isolated from 1730 mL of smooth muscle cell conditioned serum-free medium on CM-Sepharose. Proteins were eluted at 12 mL/h and 30-min fractions collected. The solid line shows protein absorbance at 280 nm. Aliquots of the fractions indicated were assayed for monocyte chemotaxis activity (■). Bovine serum albumin (M_r 65 000) and cytochrome *c* (M_r 12 700) were used as markers. The horizontal bar indicates fractions pooled for further separation.

(*g*) *Amino Acid Composition.* Samples of the purified protein material were lyophilized and hydrolyzed in 0.5 mL of 6 N HCl under vacuum at 110 °C for 24 h. Amino acid composition was determined on a Beckman System 7300 high-performance analyzer.

RESULTS

Purification of SMC-CF. The procedure adopted for the isolation of SMC-CF from baboon smooth muscle cell conditioned medium was based on our initial observations that the monocyte chemotactic activity is associated with a low molecular weight, basic protein. In addition, pilot studies indicated that the activity is stable to both low pH and lyophilization, thus allowing the use of volatile acid buffers in some separation steps. The sequence of steps leading to the isolation of SMC-CF and its recovery is summarized in Table I.

Initial isolation of the chemotactic activity in the cell-conditioned medium was carried out on CM-Sepharose. At pH 5.5 and reduced ionic strength, 70–80% of the activity bound the ion-exchange gel and was eluted in a high-salt buffer (0.05 M sodium acetate, pH 5.5, containing 1 M NaCl). The eluate was dialyzed against 1 M acetic acid (adjusted to pH 3.5 with ammonium hydroxide) to reduce sodium and chloride ion concentrations and lyophilized.

The chemotactic activity in the lyophilized material was further purified by gel permeation chromatography on Sephacryl S-200 in 1 M acetic acid. As shown in Figure 1, the activity eluted from the column as a single major peak with an elution volume similar to that of cytochrome *c* (M_r 12 500). An 80-fold purification of SMC-CF was obtained by gel permeation chromatography, with only modest losses in yield.

Active fractions from the previous isolation step were pooled (volume approximately 40 mL), lyophilized, and then separated by ion-exchange HPLC on a TSK SP-5-PW column equilibrated in 0.05 M sodium phosphate, pH 7.0. The lyo-

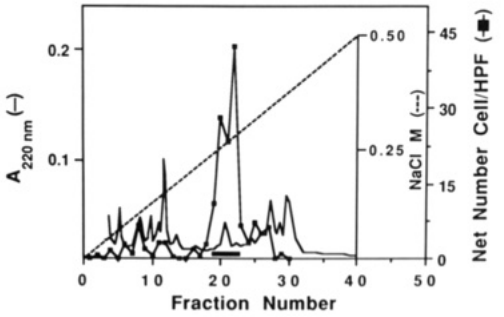

FIGURE 2: Ion-exchange HPLC on TSK SP-5-PW. Pooled active fractions obtained by gel permeation chromatography were dissolved in approximately 0.5 mL of 0.05 M sodium phosphate, pH 7.0, and applied to a 75 × 7.5 mm TSK SP-5 PW column equilibrated in the same buffer. Bound proteins were eluted at 1 mL/min in a linear 40-min gradient of 0–0.5 M NaCl in 0.05 M sodium phosphate, pH 7.0. The eluate was monitored at 220 nm, and 1-min fraction were collected. Aliquots of the indicated fractions were assayed for chemotactic activity (■) and the active fractions indicated by the horizontal bar pooled for further separation.

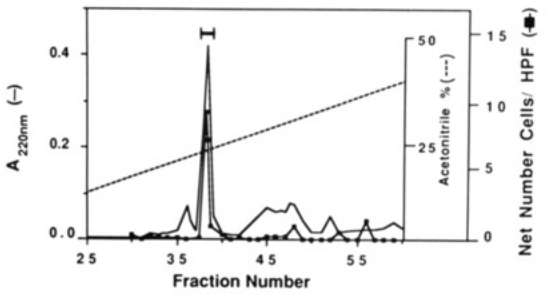

FIGURE 3: Purification of SMC-CF by reversed-phase HPLC. Pooled active fractions fron the ion-exchange HPLC step were applied directly to a 250 × 4.6 mm C_4 column in 0.1% TFA by multiple 1-mL injections. Elution was achieved with a linear 50-min gradient of 0–50% acetonitrile in 0.1% TFA. Absorbance was monitored at 220 nm (solid line). Aliquots of the indicated fractions were tested for monocyte chemotaxis activity (■). The horizontal bar indicates fractions pooled for rechromatography.

philized protein was dissolved and applied to the column in the same buffer. Bound proteins were eluted in a linear (0–0.5 M) gradient of NaCl in 0.05 M sodium phosphate, pH 7.0. As shown in Figure 2, chemotactic activity eluted in a single major peak in 0.2–0.25 M NaCl. The activity corresponded to a symmetrical peak of absorbance at 220 nm.

Final purification of SMC-CF was achieved by reversed-phase HPLC. Active fractions obtained by ion-exchange chromatography were pooled and applied to a C_4 column equilibrated in 0.1% TFA and eluted in a linear (0–50%) gradient of acetonitrile in 0.1% TFA. The total volume of the pooled activity (3–4 mL) was applied in successive 1-mL injections before the gradient was started. Chemotactic activity eluted in approximately 30% acetonitrile as a single sharp peak that corresponded to a separate peak of absorbance at 220 nm (Figure 3). Fractions under this peak were pooled and diluted with an equal volume of 0.15% heptafluorobutyric acid (HFBA) for rechromatography.

Rechromatography of SMC-CF was carried out on the C_{18} support with elution in a linear (27–55%) gradient of acetonitrile in 0.15% HFBA. Monocyte chemotactic activity copurified with a single distinct absorbance peak at approximately 50% acetonitrile (Figure 4). Active fractions under this peak were pooled and analyzed further. The purification of SMC-CF was approximately 15000-fold with a yield of 10% of the initial total activity.

In the quantitation of the recovery and purification of SMC-CF (Table I), certain approaches were made to reduce

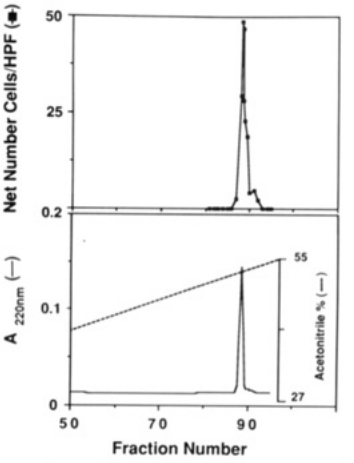

FIGURE 4: Rechromatography of SMC-CF on reversed-phase HPLC. Active fractions obtained from the C_4 support were pooled and diluted with 1 volume of 0.15% HFBA, applied to a 250 × 4.6 mm C_{18} column equilibrated in 0.15% HFBA/27% acetonitrile, and eluted with a linear 30-min gradient of 27–55% acetonitrile in 0.15% HFBA. Absorbance was monitored at 220 nm (solid line). Aliquots of the indicated fractions were tested for monocyte chemotaxis activity (■).

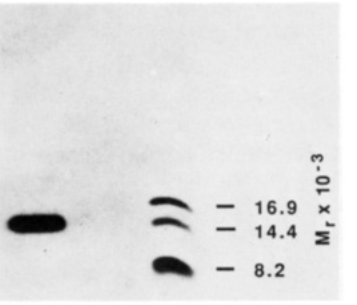

FIGURE 5: $NaDodSO_4$–polyacrylamide gel electrophoresis of isolated SMC-CF. Approximately 400 ng of the preparation obtained from reversed-phase HPLC on the C_{18} support (Figure 4) were dissolved in 10 μL of sample buffer containing β-mercaptoethanol and boiled for 1 min. Following electrophoresis, the gel was fixed and stained with silver as described under Experimental Procedures. Molecular weight markers were myoglobin, M_r 16900 and its cyanogen bromide peptides, M_r 14400, 8200, 6200, and 2500 (Sigma Chemical Co.). The M_r 6200 and 2500 peptide fragments were not observed at the concentration of the standards used.

inaccuracy. In particular, in order to eliminate the influence of donor variability in the monocyte chemotaxis assay, chemotactic activity in the pooled fractions obtained at each isolation step was estimated in a single assay at the end of the purification procedure by using cells from a single donor. In addition, the standard curve used in the assay was established with the purified protein obtained in that particular experiment. However, the possible presence in the semipurified preparations of factors that may enhance or inhibit chemotaxis in the filter assay indicates that the values presented in Table I are only approximate. By use of this procedure, approximately 0.5–1 μg of purified SMC-CF (approximately 10% yield) may be isolated from 1 L of the smooth muscle cell conditioned medium.

Characterization of *SMC-CF*. The purity of the final preparation was analyzed by $NaDodSO_4$–polyacrylamide gel electrophoresis. Electrophoresis of the protein under reducing conditions revealed a single major protein band of M_r 14500 (Figure 5). The same pattern was observed when the protein was electrophoresed under nonreducing conditions. Monocyte chemotactic activity could be eluted with the band observed in unreduced, non-silver-stained gels.

The addition of cycloheximide to the smooth muscle cell cultures was previously shown to inhibit the production of

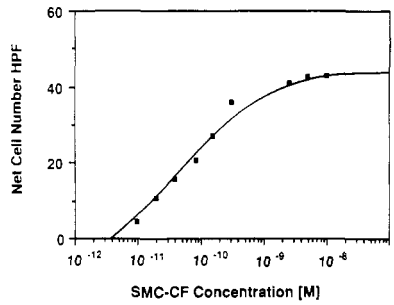

FIGURE 6: Monocyte chemotactic activity of purified SMC-CF. Monocyte chemotaxis was carried out by using Boyden blind-well chemotaxis chambers and polycarbonate 5-μm pore filters as described under Experimental Procedures. Purifeid SMC-CF (concentration determined by amino acid composition analysis) was diluted in M199 containing 2 mg/mL BSA and placed below the filters. Values obtained with medium alone were subtracted from the experimental values. Each point represents the mean from duplicate assays.

chemotactic activity, indicating the requirement for protein synthesis for its accumulation in the medium (Valente et al., 1984b). To confirm that the smooth muscle cells synthesize and secrete the chemoattractant and that it is not a modified product of the fetal bovine serum in which the cells are grown, cultures were incubated in [^{35}S]methionine/[^{35}S]cysteine and the protein was isolated from the labeled medium. Separation of the material by NaDodSO$_4$–polyacrylamide gel electrophoresis followed by staining and autoradiography revealed a single band on the autoradiogram coincident with the single silver-stained band (M_r 14 500) observed on the gel (data not shown), indicating SMC-CF protein to be synthesized by the smooth muscle cells.

The isolated protein material contained potent chemotactic activity for human peripheral blood monocytes (Figure 6). Assuming a molecular mass of 14 500, maximal stimulation of monocyte migration was obtained with approximately 5 nM SMC-CF, while half-maximal stimulation was seen at a concentration of 0.1 nM.

The amino acid composition of purified SMC-CF is shown in Table II. Values represent the average of results from three separate preparations, which were calculated as residues per molecule (on the basis of a molecular mass of 14 500), and adjusted to the nearest integer. The content of cysteine and tryptophan was not determined. Baboon aortic smooth muscle cells in culture express the genes for both the A and B polypeptide chains of platelet-derived growth factor (PDGF) (see Discussion), a basic protein with potent mitogenic activity for certain mesenchymal cells. It has been reported that PDGF also demonstrates chemotactic activity for a number of cell types, including peripheral blood monocytes. A comparison of the amino acid composition of SMC-CF with those of the mature A and B chains of PDGF (deduced from sequence data) suggests that it is not derived from either of these polypeptides. In particular, SMC-CF contains more aspartic acid/asparagine and fewer valine residues than either of these polypeptides. In addition, it is distinguishble from PDGF B by the presence of tyrosine and from PDGF A by the presence of methionine.

Table II: Amino Acid Composition of Baboon SMC-CF and Human PDGF-A and PDGF-B

amino acid	residues/molecule		
	SMC-CF[a]	PDGF-A[b]	PDGF-B[c]
Asx	13	6	7
Thr	7	9	9
Ser	9	9	4
Glx	15	15	13
Pro	10	10	7
Gly	6	3	2
Ala	9	6	9
Cys	ND[d]	8	8
Val	8	14	12
Met	1	0	1
Ile	9	4	7
Leu	5	6	7
Tyr	2	3	0
Phe	4	1	3
His	3	3	1
Trp	ND[d]	1	1
Lys	13	14	7
Arg	7	13	11

[a] Values based on three separate preparations (0.5, 2.0, and 5.0 μg) hydrolyzed unreduced for 24 h. Calculated by assuming M_r = 14 500 and adjusting to the nearest integer. [b] Residues per peptide of mature PDGF-A (125 amino acids) derived from sequence data of Betsholtz et al. (1986). [c] Residues per peptide of mature PDGF-B (109 amino acids) derived from sequence data of Collins et al. (1985). [d] ND = not determined.

Discussion

This paper provides a description of the purification of a protein chemoattractant for peripheral blood monocytes from the conditioned medium of mammalian vascular cells in culture. In previous studies (Valente et al., 1984b), we reported the partial characterization of a baboon aortic medial smooth muscle cell derived chemotactic factor (SMC-CF) and showed it to be associated with a basic (pI ~10) protein of low molecular mass. Here we describe the purification to homogeneity of microgram quantities of this potent monocyte chemoattractant and detail its further characterization. Purification (15 000-fold) from conditioned serum-free medium was achieved by concentration from whole medium on CM-Sepharose, gel permeation chromatography on Sephacryl S-200, ion-exchange HPLC on a TSK SP-5-PW column, and finally reversed-phase HPLC on C_4 and C_8 supports. Elution of SMC-CF activity from Sephacryl S-200 indicated a protein of approximately 12 000 molecular mass. On analytical NaDodSO$_4$–polyacrylamide gel electrophoresis, SMC-CF migrated as a single-chain polypeptide of M_r 14 500. Synthesis of this protein by the smooth muscle cells was confirmed by metabolic labeling with [^{35}S]methionine/[^{35}S]cysteine and autoradiography.

Smooth muscle cells in culture secrete a number of proteins that have been shown to be potentially chemotactic for peripheral blood monocytes. Among these are the connective tissue proteins procollagen, tropoelastin, and fibronectin (Muir et al., 1976; Postlethwaite & Kang, 1976; Burke & Ross, 1979; Hunninghake et al., 1981; Norris et al., 1982). Under certain circumstances they may also synthesize and secrete the growth competence factor PDGF (Seifert et al., 1984; Nilsson et al., 1985; Sejersen et al., 1986; Walker et al., 1986).

PDGF is a basic, dimeric (M_r ~30 000) polypeptide hormone that has been implicated as a mediator in both human and experimental atherosclerosis (Ross, 1986). In addition to stimulating mitogenesis in mesenchymal cells (Ross et al., 1974; Antoniades et al., 1975; Westermark & Wasteson, 1975), PDGF has been reported to be chemotactic for fibroblasts (Seppä et al., 1982), smooth muscle cells (Grotendorst et al., 1981), neutrophils, and monocytes (Deuel et al., 1982). There is evidence that mitogenesis and chemotaxis are mediated by different structural determinants on the PDGF molecule, since only the dimeric form of PDGF is mitogenic, while both the dimeric and monomeric forms are reported to be chemotactic for monocytes (Deuel et al., 1982). Recent studies also indicate that fragments of PDGF resulting from hydrolysis with some proteases retain their chemotactic activity

(Senior et al., 1985). Although the cultured baboon smooth muscle cells used in these studies transcribe the genes for both the A and B polypeptide chains of PDGF, they do not synthesize and secrete detectable dimeric mitogenic PDGF proteins. They do, on the other hand, secrete a low molecular weight ($M_r \sim 12000$) monomeric protein that is recognized by specific antibodies to human PDGF (Valente et al., 1988). There is a possibility, therefore, that SMC-CF may be derived in some manner from PDGF. This appears to be unlikely, however, since a comparison of the amino acid composition of SMC-CF with those of the mature A and B polypeptide chains of PDGF reveals several important differences in the content of some amino acids (Table II).

The amino acid composition of SMC-CF also suggests that it is not derived from elastin since it contains approximately three residues per molecule of histidine, an amino acid observed rarely in elastin obtained from mammalian vascular tissue (Rosenbloom, 1984). Similarly, it can be distinguished from the *N*-propeptide and the helical domains of types I and III collagen simply on the basis of composition alone (Horlein et al., 1979; Brandt et al., 1984; Kühn, 1984). On the other hand, such distinctions cannot be made on this basis with either the *C*-propeptide domain of collagen (Fuller & Boedtker, 1979) or with fibronectin (Vuento et al., 1977). It is worth noting, however, that the concentrations of both the collagen and fibronectin cleavage products required for optimum monocyte chemotactic activity (Postlethwaite & Kang, 1976; Norris et al., 1982) are 10–100-fold higher than those reported here for SMC-CF.

The role that SMC-CF may play in both the maintenance of the integrity of the normal artery and in the pathogenesis of atherosclerosis remains to be determined. Monocytoid cells of peripheral blood origin can be observed in the normal aortic intima of a number of species (Geer, 1965; Lee et al., 1970; Joris et al., 1979) and probably represent a basal or constitutive level of recruitment that is part of the normal immunological surveillance of the vascular tissue. Regulation of this recruitment activity may well be a major function of the smooth muscle cell derived monocyte chemotactic factor. Enhanced intimal recruitment of monocytes observed in experimental and human atherosclerosis may thus result from the increased secretion of SMC-CF, which in turn may be part of the response-to-injury reaction of the vascular smooth muscle cells. The successful isolation and purification to homogeneity of SMC-CF provide the basis for the further structural characterization of this protein, facilitate studies determining the factors that modulate its synthesis and secretion, and permit the further characterization of its biologic roles.

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