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Characterization of Monocyte Chemotactic Protein-1 Binding to Human Monocytes

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SUMMARY: Monocyte chemotactic protein-1 (MCP-1) stimulates chemotaxis of peripheral blood monocytes. In order to understand the biologic basis of this specific activity, binding studies of ^{125}I-MCP-1 were undertaken. MCP-1 showed saturable binding to monocytes. Scatchard analysis of the monocyte binding data indicate that there are approximately 1,600 high affinity binding sites per monocyte with a Kd = 1.1 nM. Studies with synthetic peptides constructed according to the MCP-1 amino acid sequence indicate that a synthetic peptide, MCP-1[13-35], stimulates monocyte migration and competes with native MCP-1 for binding sites. Inhibition of MCP-1 binding was tested with chemotactic connective tissue proteins. No inhibition of MCP-1 binding was observed with either collagen, elastin-derived peptides or fibronectin. These results identify a single class of unique high affinity MCP-1 binding sites that are likely to recognize a peptide domain on MCP-1 which include the amino acids within the region, 13-35.

Monocyte chemotactic protein-1 (MCP-1) is a potent chemoattractant that is specific for monocytes. It is produced by several different types of cells in vitro including vascular smooth muscle cells, endothelial cells, fibroblasts, activated monocytes/macrophages and cells derived from malignant carcinomas and sarcomas (Rev. in ref (1,2). The secretion of MCP-1 in vivo may constitute an important mechanism for stimulating monocyte migration in both normal and pathologic conditions.

The directed migration of mononuclear phagocytes in gradients of chemotactic substances is dependent upon the presence of highly specific cell surface receptors. In this study we have characterized the binding of ^{125}I-labeled MCP-1 to highly purified preparations of peripheral blood monocytes. In addition, a synthetic peptide, MCP-1[13-35], induced monocyte

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Abbreviations: MN, peripheral blood monocytes; MCP-1, monocyte chemotactic protein-1.

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chemotaxis and competed with ^{125}I-MCP-1 for high affinity binding sites, suggesting that this peptide domain directly participates in binding. In contrast, connective tissue proteins that were previously shown to be chemotactic, did not to compete with MCP-1 for binding sites.

MATERIALS AND METHODS

LABELING OF CHEMOTACTIC FACTOR. MCP-1 was purified from serum-free medium conditioned by baboon vascular smooth muscle cells as described previously (3). MCP-1 was labeled with ^{125}I using the Bolton-Hunter reagent (ICN Radiochemicals, Irvine, Ca, >2000 Ci/mmol), resulting in specific activities of 30 - 160 cpm/pg.

HUMAN LEUKOCYTE PREPARATION. Highly purified preparations of human MN were prepared by Ficoll/Hypaque gradient centrifugation and counterflow centrifugal elutriation (4). In all binding experiments, monocytes were >70% pure as assessed from Wright-Giemsa stained smears and greater than 95% viable as determined by trypan blue dye exclusion.

BINDING AND ASSAYS. Human monocytes were incubated with 0.5 - 13.0 nM ^{125}I-MCP-1 at 8°C for 3 hours. The cells were centrifuged, resuspended and separated from the remaining unbound labeled ligand by layering over 125 ul of 5% BSA, followed by centrifugation. Non-specific binding was determined in the presence of 40 fold excess unlabelled MCP-1.

SYNTHETIC PEPTIDES: Peptides were synthesized by Multiple Peptide Systems (San Diego, CA). The carboxyl terminal amino acid was synthesized as an amide. MCP-1[13-35], corresponded to amino acids 13-35 of mature MCP-1 (YNFTNRKISVQRLASYRRITSSK-amide). MCP-1[37-50], corresponded to amino acids 37-50 of mature MCP-1 (SPKEAVIFKTIVAKEI-amide). Sequences were confirmed by GC-Mass Spectrometry (Beckman Research Institute (Duarte, CA). Peptides were purified following synthesis by reverse phase HPLC.

CONNECTIVE TISSUE PROTEINS. Human collagen type 1 (from fetal membrane), >95% pure, was obtained from Calbiochem, La Jolla, CA. Fibronectin was isolated from the fresh plasma of healthy donors by affinity chromatography on gelatin-Sepharose and ion exchange chromatography on DEAE-Sepharose essentially as described (5). Peptides of elastin were prepared by partial hydrolysis of bovine ligamentum nuchae elastin.

RESULTS

Pure preparations of MCP-1 were labeled with the Bolton-Hunter reagent (30 - 160 cpm/pg). When ^{125}I-MCP-1 was analyzed by SDS-PAGE and autoradiography, a single band was observed with Mr = 14,400. The ability of both the labeled and unlabeled protein to attract monocytes in the chemotactic filter assay was identical, with optimum activities being observed in the range of 1 - 10 ng/ml (data not shown). Binding experiments demonstrate the presence of high affinity binding sites typical of ligand-receptor interactions. Saturable binding was observed in experiments carried out at 8°C (Fig. 1A). Scatchard analysis of the binding data indicate the presence of a single class of high affinity binding sites (Fig. 1B). The Kd was calculated to be 1.1 nM, with 1,600 high affinity binding sites per monocyte.

Synthetic peptides were constructed to identify the binding region of MCP-1. MCP-1[13-35] stimulated a dose-dependent increase in monocyte migration, with a maximum response

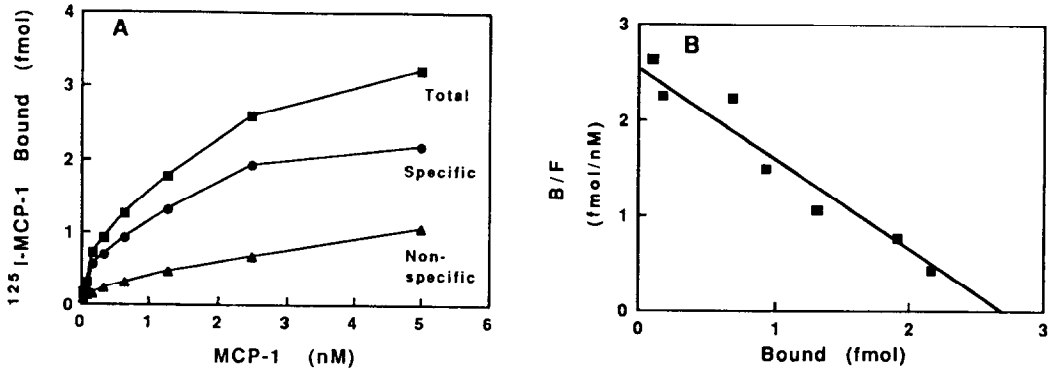

Figure 1. ^{125}I MCP-1 binding to human monocytes. Increasing concentrations of ^{125}I MCP-1 (94 cpm/pg) were added to freshly isolated human monocytes and incubated for 3 hours at 8°C. Non-specific binding was determined in the presence of 40 fold excess unlabelled MCP-1. **A.** Saturation binding. **B.** Scatchard analysis of binding in A.

occurring at 20-50 uM (Figure 2). Another synthetic peptide, MCP-1[37-50], had little or no chemotactic activity when tested at similar concentrations (data not shown). Since MCP-1[13-35] demonstrated chemotactic activity, its capacity to inhibit the binding of native MCP-1 was then tested (Fig. 3). MCP-1[13-35] inhibited the binding of ^{125}I-MCP-1 at concentrations of 10-100 uM. This is nearly identical to the concentrations required to stimulate monocyte migration.

Many connective tissue proteins, or their degradation products, display varying degrees of chemotactic activity for MN (5,6,7). Elastin peptides derived from bovine ligamentum nuchae elastin, human type 1 collagen and human plasma fibronectin stimulated migration of human

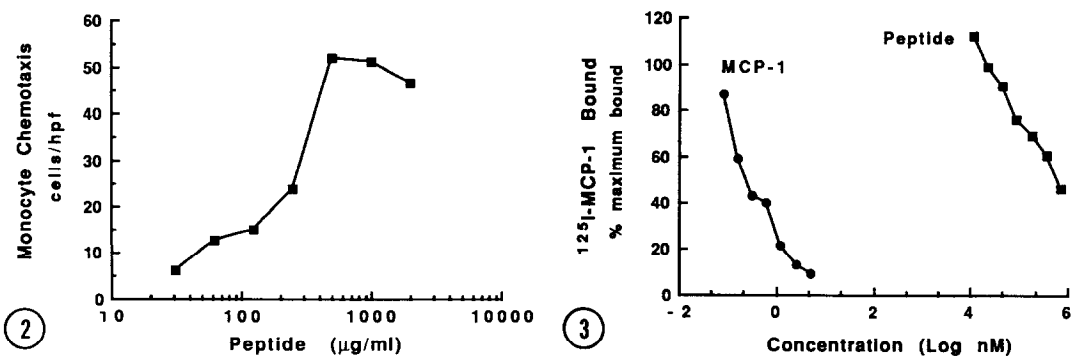

Figure 2. Synthetic peptide, MCP-1[13-35] stimulates monocyte migration. MCP-1[13-35], Mr = 2,789, was tested for stimulating the migration of human peripheral blood monocytes in a Boyden chamber assay. Each value represents the number of monocytes per ten high power fields for duplicate samples, which varied by less than 10 percent from the mean.

Figure 3. Synthetic peptide, MCP-1[13-35] competes with native ^{125}I MCP-1 for binding sites on human monocytes. ^{125}I MCP-1 was incubated with human peripheral blood monocytes in the presence of MCP-1[13-35] or native unlabelled MCP-1. Bound ^{125}I MCP-1 was separated from free ligand and counted. Each value represents the mean of duplicate samples which varied less than 10 % from the mean.

Table 1

Competition for ^{125}I MCP-1 Binding to Human Monocytes by Connective Tissue Proteins

PROTEIN	CONCENTRATION (nM)	^{125}I MCP-1 BOUND % uninhibited control (sd)
MCP-1	20.0	27.1 (3.1)
	2.0	47.6 (5.7)
	0.2	82.3 (8.3)
Elastin peptides	20.0	103.6 (7.4)
	2.0	94.2 (20.2)
	0.2	93.7 (8.6)
Collagen	20.0	87.5 (14.8)
	2.0	96.8 (7.9)
	0.2	93.7 (8.7)
Fibronectin	20.0	93.8 (16.8)
	2.0	105.1 (15.9)
	0.2	95.9 (7.9)

^{125}I MCP-1 (2 nM) was incubated with unlabelled MCP-1, elastin peptides, collagen or fibronectin (0-20 nM) for two hours at 25°C in the presence of human monocytes (>75%). Results are expressed as the percent bound ^{125}I-MCP-1 for each sample versus the uninhibited control. Each value represents the mean of triplicate samples.

monocytes when tested at 5 ug/ml (data not shown). None of the connective tissue proteins tested competed for the binding of ^{125}I-MCP-1 to the monocytes (Table 1) even when present in 10-fold molar excess. This indicates that MCP-1 stimulated monocyte chemotaxis is mediated through cell surface receptors distinct from those responsible for collagen, elastin and fibronectin-stimulated chemotaxis.

DISCUSSION

The studies described in this report were undertaken to identify and characterize specific high affinity binding sites for MCP-1 on MN. Analysis of the binding data indicate that ^{125}I-MCP-1 binds to high affinity, low capacity binding sites (1,600 per cell) with a Kd of approximately 1.1 nM. Interestingly, no high affinity binding sites were observed on purified preparations of either human lymphocytes or polymorphonuclear leukocytes (data not shown), cell types which were previously shown not to respond to MCP-1 (8). Yoshimura and Leonard have recently described MCP-1 binding to monocytes (9). They calculate approximately 1,700 binding sites per cell with a Kd of 1.9 nM. These results are similar to those reported here.

MCP-1 is structurally related to a number of inflammatory cytokines (10). The greatest degree of homology is with JE, a growth factor inducible gene, which is thought to be the murine equivalent to MCP-1 (11). MCP-1 and JE have four half-cystine residues conserved at the same location. This allows for the potential formation of two intrachain loops. Synthetic

peptides were constructed according to the amino acids that would form these loops; residues 13-35 and 37-50. In addition, MCP-1[13-15] includes the region in which MCP-1 from human cells, MCP-1 from baboon vascular smooth muscle cells and JE share the greatest degree of homology. MCP-1[13-15] stimulated monocyte migration in a dose-dependent relationship, while MCP-1[37-50] did not. Furthermore, MCP-1[13-15] inhibited the binding of MCP-1 in the same concentrations that it stimulated monocyte migration. This result suggests that this domain directly participates in MCP-1 binding to its cognate receptor. That high concentrations of MCP-1[13-35] are required to stimulate monocyte chemotaxis and inhibit MCP-1 binding indicate that considerations other than the primary structure of this region affect ligand-receptor interactions. These could include the formation of disulfide bonds or the influence of other regions of the molecule that potentially affect tertiary structure.

When tested in the competition binding assay, chemotactically active preparations of human type I collagen, bovine elastin-derived peptides and human plasma fibronectin all failed to inhibit the binding of ^{125}I-MCP-1 to the monocyte cell surface. Thus, these connective tissue proteins stimulate monocyte chemotaxis through cell surface receptors distinct from those responsible for MCP-1 chemoattractant activity. In addition, studies with platelet derived growth factor and transforming growth factor beta indicate that neither of these proteins compete with MCP-1 for high affinity binding sites (data not shown). These results are consistent with recent structural data which indicate that MCP-1 belongs to a novel family of proteins unrelated to the above described proteins (3,10).

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REFERENCES

1. Graves, D. T. and Valente, A. J. (1991) Biochem. Pharmacol. 41:333-337.

2. Leonard, E. J. (1990) Immunology Today 11:87-91.

3. Valente AJ, Graves DT, Vialle-Valentin CE, Delgado R and Schwartz CJ (1988) Biochemistry 27:4162-4168.

4. Kelley, J. L., Rozek, M. M., Suenram, C. A. and Schwartz C J (1987) Exp. Mol. Pathol. 46:266-278.

5. Norris, D. A., Clark, R. A. F, Swigart, L. M., Huff, J. C., Weston, W. L. and Howell, S. E. (1982) J. Immunol. 129:1612-1618.

6. Postelthwaite, A. E. and Kang (1976) J. Exp. Med. 143:1299-1307.

7. Hunninghake, G. W., Davidson, J. M., Rennard, S., Szapiel, S., Gadek, J. E. and Crystal, R. G. (1981) Science 212:925-927.

8. Valente, A. J., Fowler, S. R., Sprague, E. A., Kelley, J. L., Suenram, A. C. and Schwartz, C. J. (1984) Am J Pathol 117:479-485.

9. Yoshimura, T. and Leonard, E. J. (1990) J Immunol. 145, 292-297.

10. Yoshimura, T., Yuhki, N., Moore, S. K., Appella, E., Lerman, M. I. and Leonard, E. J. (1989) FEBS LETTERS 244:487-493.

11. Rollins, B. J., Stier, P., Ernst, T. E. and Wong, G. G. (1989) Mol. Cell. Biol. 9:4687-4695.