Purification, Sequence Analysis, and Biological Characterization of a Second Bovine Monocyte Chemotactic Protein-1 (Bo MCP-1B)[†,‡]

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ABSTRACT: Madin Darby bovine kidney (MDBK) cells were used as a source to identify novel bovine chemotactic factors for granulocytes and monocytes. A major bovine granulocyte chemotactic protein (GCP-2) has previously been isolated. A novel bovine monocyte chemotactic protein (bo MCP) was produced on MDBK cells stimulated with phorbol ester. The 14-kDa protein was purified to homogeneity by adsorption to controlled pore glass, heparin affinity chromatography, cation-exchange FPLC, and RP-HPLC. The amino acid sequence of the NH_2-terminally blocked protein was determined by Edman degradation using proteolytic fragments. The primary structure of the bo MCP, characterized by four conserved cysteines, allowed classification of the protein within the C-C chemokine family. Bo MCP-1B was most related to known human and bovine MCPs. Compared to bovine MCP-1 and MCP-2, the protein consists of 84% and 53% identical amino acids, respectively. Since this bo MCP was also most homologous to human and animal MCP-1, it was designated bo MCP-1B. The minimal effective dose of bo MCP-1B for monocyte chemotactic activity was 0.2 nM. The maximal migration index, reached at 2 nM, was comparable to that of natural human MCP-1. Furthermore, bo MCP-1B was found to be capable of stimulating β-glucuronidase release from monocytes. In contrast, bo MCP-1B was not chemotactic for neutrophilic and eosinophilic granulocytes. By its biological and biochemical characteristics, bo MCP-1B has to be considered as an authentic additional MCP-1 chemokine. The existence of a possible human counterpart for this novel MCP-1B still needs to be elucidated.

In addition to known leukocyte chemotactic molecules such as the bacterial formylated tripeptide formylmethionyl-leucylphenylalanine (fMLP), the anaphylatoxin C5a and the leukotriene B4, proteins of a new family of chemotactic cytokines have been identified during the last decade. Four cysteine residues are conserved in all these leukocyte-specific chemoattractants belonging to this group of 5−15-kDa proteins. These chemotactic cytokines, or chemokines, have been subdivided into two subfamilies depending on whether the first two cysteines are adjacent (C-C chemokines) or not (C-X-C chemokines).

The human chemokines belonging to the C-X-C branch such as IL-8, GRO, NAP-2, ENA-78 [reviewed in Oppenheim et al. (1991), Baggiolini et al. (1994), and Van Damme 1994], and the recently described human and bovine granulocyte chemotactic protein GCP-2 (Proost et al., 1993a) are found to be mainly active on granulocytes. The genes of human C-X-C chemokines are located on chromosome 4, whereas human C-C chemokine-genes are located on chromosome 17. Recently, we discovered two novel human monocyte chemotactic proteins, MCP-2 and MCP-3 (Van Damme et al., 1992), which are structurally most related to the C-C chemokine human MCP-1 (62% and 71% identical amino acids, respectively). These three MCPs seem to form a separate subfamily within the C-C chemokine branch.

The C-C chemokines are active on different types of leukocytes, such as monocytes for MCP-1, MCP-2, MCP-3, RANTES, I-309, MIP-1α, and MIP-1β (Yoshimura et al., 1989; Van Damme et al., 1989, 1992; Schall et al., 1990; Miller & Krangel, 1992; Wang et al., 1993); eosinophils for MIP-1α, RANTES, MCP-2, and MCP-3 (Kameyoshi et al., 1992; Rot et al., 1992; Alam et al., 1993; Noso et al., 1994); basophils for MCP-1, MCP-2, MCP-3, MIP-1α, and RANTES (Alam et al., 1992a,b, 1994; Kuna et al., 1992; Dahinden et al., 1994); and specific lymphocyte subsets for MIP-1α, MIP-1β, and RANTES (Schall et al., 1990; Taub et al., 1993).

For both fundamental and experimental reasons, chemokines of various species have been studied in detail. Rat (Yoshimura et al., 1991), rabbit (Yoshimura & Yuhki, 1991), mouse (Rollins et al., 1988) and cow (Wempe et al., 1991) MCP-1 have been sequenced, and recently guinea pig and sheep MCP-1 (Yoshimura, 1993), mouse MCP-3/MARC (Kulmburg et al., 1992; Thirion et al., 1994), and cow MCP-2 (Wempe et al., 1994) have been added to the growing list of identified MCP molecules. The only bovine C-X-C chemokines known so far are PF-4 (Ciaglowski et al., 1986) and GCP-2 (Proost et al., 1993b). We here report the isolation and protein sequence of a new bovine C-C chemokine active on monocytes.

MATERIALS AND METHODS

Induction of Chemokines on Bovine Kidney Cells. Madin Darby bovine kidney cells (MDBK[1] cells, ATCC CCL22) were grown in 24-well plates in Eagle's minimum essential medium with Earle's salts (EMEM, 1 mL/well) containing 10% calf serum (Gibco, Paisley, Scotland). After 1 week the medium was removed from confluent cultures, and the cells

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[‡] The bo MCP-1B protein sequence is available from the EMBL Data Library under accession number P80343.

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were incubated with 1 mL of fresh EMEM containing 2% calf serum and different concentrations of one of the following inducers: PMA (phorbol 12-myristate 13-acetate, Sigma, St. Louis, MO), double-stranded RNA polyriboinosinic:polyribocytidylic acid (poly rI:rC, P.-L. Biochemicals, Inc., Milwaukee, WI), purified natural human interleukin-1β (IL-1β; Van Damme et al., 1988), lipopolysaccharide (LPS from *Escherichia coli* 0111:B4, Difco, Detroit, MI), and vesicular stomatitis virus [VSV, $10^{8.3}$ plaque-forming units/mL (PFU/mL) on Vero cells (Heremans et al., 1980)]. After 48 h of induction, cell supernatants of stimulated and nonstimulated cells were harvested and kept frozen at −20 °C until use.

Production and Purification of Bovine Monocyte Chemotactic Factors. Confluent MDBK monolayers (175 cm^2, Nunc, Roskilde, Denmark) were washed and stimulated for 48 h with serum-free EMEM containing 10 ng/mL PMA. Conditioned medium from three consecutive PMA inductions was pooled (3 L), concentrated, and partially purified by adsorption to controlled-pore glass (CPG-10-350, Serva, Heidelberg, Germany). Chemotactic activity was eluted with 0.3 M glycine/HCl, pH 2.0, and loaded on a heparin−Sepharose (CL-6B, Pharmacia, Uppsala, Sweden) column. After being washed with the equilibrating buffer (50 mM Tris and 50 mM NaCl, pH 7.4), the chemotactic activity was eluted in a linear NaCl gradient (0.05−2 M) in 50 mM Tris, pH 7.4. The chemotactic activity, eluted from the heparin−Sepharose column, was further purified by Mono S cation-exchange fast protein liquid chromatography (FPLC, Pharmacia) using 50 mM formate, pH 4.0 (1 mL/min). Proteins were eluted in a linear NaCl gradient (0−1 M) in 50 mM formate, pH 4.0. The final purification step consisted in C-8 RP-HPLC. FPLC fractions containing chemotactic activity were injected on a C-8 Aquapore RP-300 column (Applied Biosystems Inc., Foster City, CA) equilibrated with 0.1% trifluoroacetic acid (TFA) in H_2O. Proteins were eluted with an acetonitrile gradient (solvent B: 80% acetonitrile and 0.1% TFA in H_2O) at 0.4 mL/min. Absorbance was monitored at 220 nm, and 400 μL fractions were collected.

Protein Determination and SDS−PAGE. Protein concentrations were measured with the Bio-Rad (Bio-Rad Laboratories, Richmond, CA) Coomassie blue G-250 binding assay (Bradford, 1976). Fractions from heparin−Sepharose chromatography, cation-exchange FPLC, and RP-HPLC were checked for purity by SDS−PAGE on a linear gradient (10−25%) polyacrylamide gel, and proteins were silver-stained. The relative molecular mass markers (Bio-Rad) phosphorylase b (M_r 92 500), BSA (M_r 66 200), ovalbumin (M_r 45 000), carbonic anhydrase (M_r 31 000), soybean trypsin inhibitor (M_r 21 500), and lysozyme (M_r 14 400) and the low relative molecular mass marker (Pierce Chemical Co., Rockford, IL) aprotinin (M_r 6500) were used.

Purification of Leukocytes. Human monocytes and granulocytes were isolated from heparinized peripheral blood from single donors. Sedimentation for 30 min in hydroxyethyl starch (Plasmasteril, Fresenius AG, Bad Homburg, Germany) was used to remove erythrocytes. Mononuclear cells and granulocytes were further separated by gradient centrifugation for 30 min at 400g on Ficoll−sodium metrizoate (Lymphoprep, Nyegaerd, Oslo, Norway). For monocyte chemotaxis experiments the total mononuclear cell fraction was used. Remaining erythrocytes in the granulocyte pellet were eliminated by lysis in double-distilled water for 30 s. A final purity of more than 95% was reached for the mononuclear cell fraction, whereas the neutrophil granulocyte fraction contained 10−20% eosinophils.

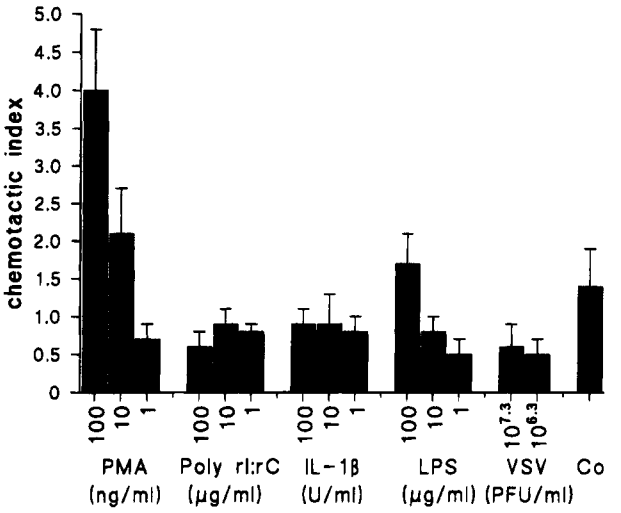

FIGURE 1: Production of monocyte chemotactic activity by bovine kidney cells. MDBK cells were induced with different concentrations of PMA, Poly rI:rC, IL-1β (U, international units), LPS, and VSV (PFU, plaque forming units) or were left untreated (Co). Monocyte chemotactic activity in the supernatant was measured with the Boyden chamber assay, and the mean chemotactic index (±SEM) of three independent experiments is shown.

β-Glucuronidase Release Assay. β-Glucuronidase activity was measured as previously described (Schröder et al., 1987). After a preincubation with 5 μg/mL cytochalasin B for 10 min at 37 °C, 10^6 purified mononuclear cells or granulocytes in 100 μL of HBSS+HSA [Hanks' balanced salt solution supplemented with 1 mg/mL human serum albumin (HSA)] were incubated with 100 μL of test sample for 1 h at 37 °C. For maximum enzyme release cells were lysed with 100 μL of 0.2% (v/v) Triton X-100, and incubation in HBSS+HSA was taken as a negative control. After incubation the cells were centrifuged at 1000g for 10 min at 4 °C, and 100 μL of supernatant was incubated with 100 μL of 0.01 M *p*-nitrophenyl β-D-glucuronide in 0.1 M sodium acetate, pH 4.0, for 18 h at 37 °C. The reaction was stopped by adding 200 μL of 0.4 M glycine buffer, pH 10.0, and the yellow color was measured photometrically at 405 nm. β-Glucuronidase release was expressed as a percentage of the maximal release obtained with Triton X-100 lysed cells.

Assays for Chemotaxis of Cells. Monocyte and granulocyte chemotaxis was measured in a Boyden microchamber (Neuro Probe Inc., Cabin John, MD) assay as previously described (Van Damme et al., 1989). The lower compartment of the microchamber was filled with dilutions of test samples (27 μL). For monocyte chemotaxis (37 °C, 120 min) the upper compartment was filled with 2 × 10^6 cells/mL (50 μL) in HBSS+HSA. Lower and upper compartment were separated by a 5 μm pore size polyvinylpyrrolidone-treated polycarbonate membrane (Nuclepore, Pleasanton,

[1] Abbreviations: IL-8, interleukin-8; MCP, monocyte chemotactic protein; PMA, phorbol 12-myristate 13-acetate; poly rI:rC, polyriboinosinic:polyribocytidylic acid; LPS, lipopolysaccharide; VSV, vesicular stomatitis virus; MDBK, Madin Darby bovine kidney cells; EMEM, Eagle's minimum essential medium; HBSS, Hanks' balanced salt solution; HSA, human serum albumin; RP-HPLC, reverse-phase high-performance liquid chromatography; FPLC, fast protein liquid chromatography; TFA, trifluoroacetic acid.

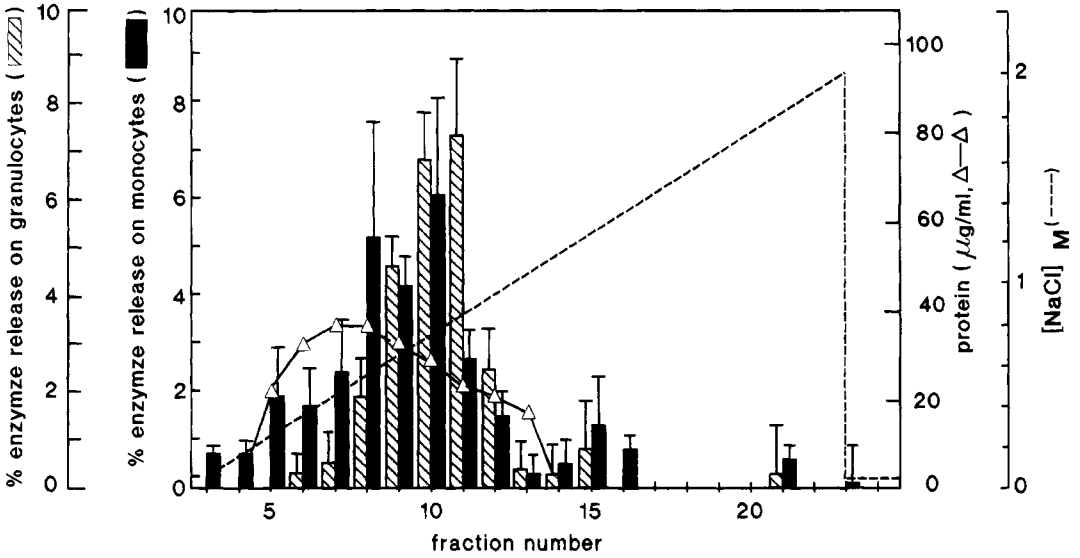

FIGURE 2: Separation of monocyte- and granulocyte-activating proteins by heparin–Sepharose chromatography using a NaCl gradient (- - -). Fractions were tested for their β-glucuronidase-releasing activity on monocytes (solid bars) and granulocytes (hatched bars) and for their protein concentration (△). β-Glucuronidase release is expressed as the mean percentage ± SEM of the maximal enzyme release obtained from Triton X-100 lysed monocytes ($n = 6$) or granulocytes ($n = 4$).

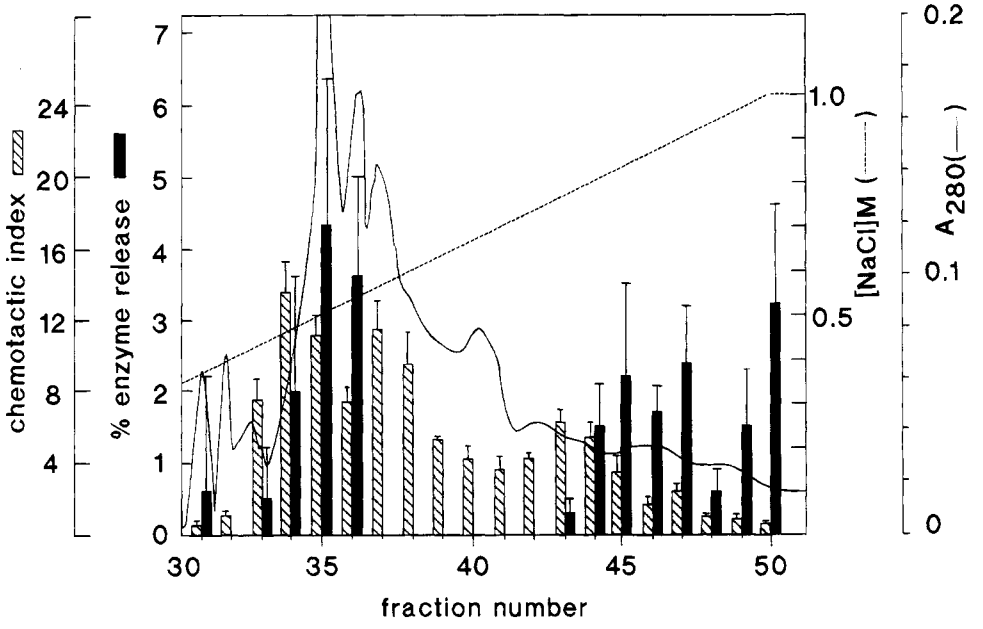

FIGURE 3: Purification of monocyte chemotactic (hatched bars) and enzyme-releasing (solid bars) activities on Mono S cation-exchange FPLC using a NaCl gradient (- - -). Absorbance was measured at 280 nm (—).

CA). Granulocyte chemotaxis (37 °C, 45 min) was performed with 10^6 cells/mL (50 μL) in the same medium using a 5 μm pore size polycarbonate (polyvinylpyrrolidone free) membrane. After fixation and staining of the filters with Diff-Quick (Harleco, Gibbstown, NJ), the samples were scored by counting the cells that had migrated through the membrane in 10 microscopic fields per well. The chemotactic index was calculated by dividing the number of cells that migrated through the membrane to the test sample by the number of cells that migrated to the control medium (HBSS+HSA). Natural human IL-8 (Van Damme, 1994) and MCP-1 (Van Damme et al., 1989) were used as reference chemoattractants for neutrophils and monocytes, respectively.

Identification of Bovine Chemotactic Activity by Protein Sequence Analysis. NH_2-terminal protein sequencing was performed by Edman degradation on a pulsed liquid (477A/120A) protein sequencer (Applied Biosystems) with on-line PTH-amino acid analysis. Cysteine residues were determined by on-filter reduction and modification with tributylphosphin and 4-vinylpyridine (Aldrich Chemical Company, Inc., Milwaukee, WI) (Andrews & Dixon, 1987).

Internal peptide fragments were prepared using different proteolytic enzymes (sequencing grade; Boehringer Mannheim, Mannheim, Germany). Four micrograms of chemotactic protein was cleaved with 0.4 μg of enzyme. Digestion was obtained with endoproteinase Lys-C (for 18 h at 37 °C in Tris/HCl buffer, 25 mM, pH 8.5, and EDTA, 1 mM), Glu-C (for 18 h at 25 °C in ammonium carbonate buffer, 25 mM, pH 7.8), or Asp-N (for 18 h at 37 °C in sodium phosphate buffer, 50 mM, pH 8.0).

In order to sequence amino termini, RP-HPLC-purified blocked proteins or blocked proteolytic fragments were pretreated with pyroglutamate aminopeptidase (sequencing grade; Boehringer Mannheim). Proteins or proteolytic

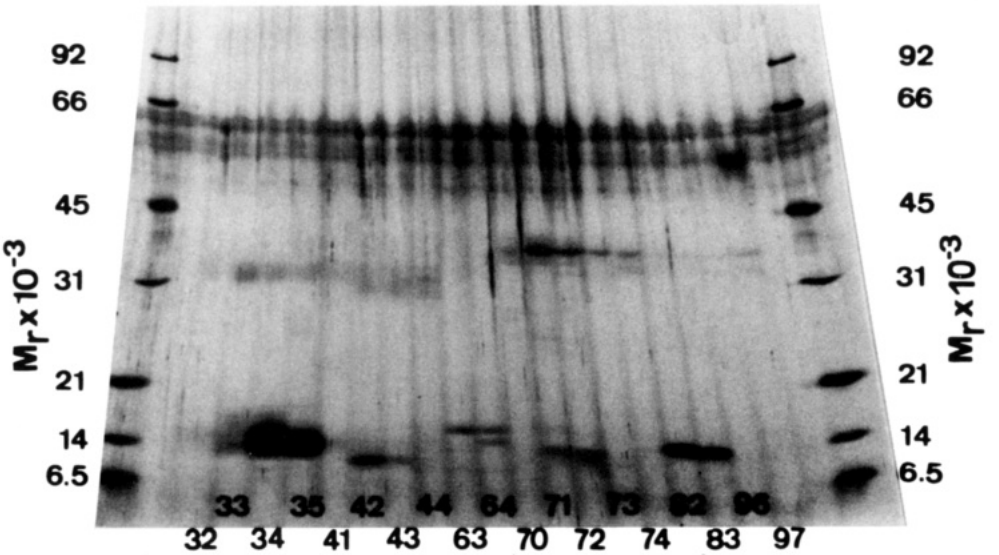

FIGURE 4: SDS−PAGE of RP-HPLC-purified bovine MCP-1B. Fractions (numbers are indicated below the gel) were loaded (20 μL/lane) on a gradient gel under reducing conditions, and proteins were visualized by silver staining. Relative molecular weight standards (M_r) are as indicated in Materials and Methods.

fragments were dissolved in 100 mM sodium phosphate buffer, pH 8.0, 10 mM Na_2EDTA, 5 mM dithioerythritol, and 5% (v/v) glycerol and incubated for 6 h at 50 °C with an enzyme:substrate ratio of 1:20 by weight.

After enzymatic incubations, the protein fragments were repurified by C-8 RP-HPLC as described for the final purification of the intact chemotactic factors. All peptides were sequenced as described above.

RESULTS

Induction of Monocyte Chemotactic Activity on MDBK Cells. Confluent MDBK monolayers were treated with different inducers or with control medium to produce chemokines (Figure 1). Conditioned media were harvested after 48 h and analyzed for monocyte chemotactic activity in the microchamber assay. Unstimulated MDBK cells produced already a detectable amount of monocyte chemotactic activity. Induction with LPS, poly rI:rC, IL-1β, or VSV did not yield a further increase in production. Significant induction of monocyte chemotactic activity, however, was obtained with the phorbol ester PMA at 100 ng/mL. PMA was therefore used for preparative inductions of monocyte chemotactic factors on MDBK cultures.

Separation and Biological Characterization of Bovine Monocyte Chemotactic and Activating Factors. MDBK-derived conditioned medium harvested after 48 h of induction with PMA was 10-fold concentrated and partially purified on controlled-pore glass and further purified on a heparin−Sepharose column. Heparin−Sepharose fractions were analyzed for β-glucuronidase release on human monocytes and granulocytes (Figure 2). The main activity for monocytes eluted from the heparin−Sepharose column between 0.5 and 0.9 M NaCl. The main peak of enzyme releasing activity for granulocytes coincided with that for monocytes. The total amount of protein measured in the eluate was about 20% of the input material. More than 60% of the amount of protein eluted in the void fractions but did not contain any measurable enzyme-releasing activity (Figure 2).

Heparin−Sepharose column fractions (0.5−0.9 M NaCl) containing activity for monocytes were pooled and further purified by Mono S cation-exchange FPLC. β-Glucuronidase-releasing activity on monocytes was separated into two parts, a major part eluting at about 0.5 M NaCl and a minor one eluting around 0.8 M NaCl (Figure 3). No effect was observed with the 0.5 M NaCl peak when enzyme-releasing activity was tested on neutrophil and eosinophil granulocytes

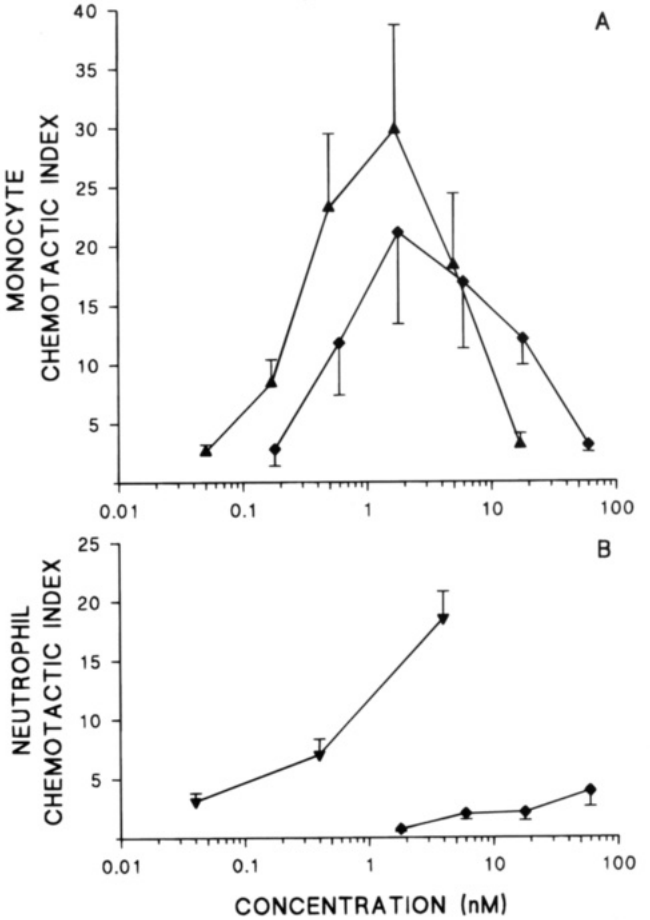

FIGURE 5: Monocyte (panel A) and neutrophil (panel B) chemotactic activity of bo MCP-1B (◆) in the Boyden chamber. Natural human MCP-1 (▲) and IL-8 (▼) are used as positive controls for monocyte and neutrophil chemotaxis, respectively. Average chemotactic indexes (±SEM) were derived from three or more independent experiments.

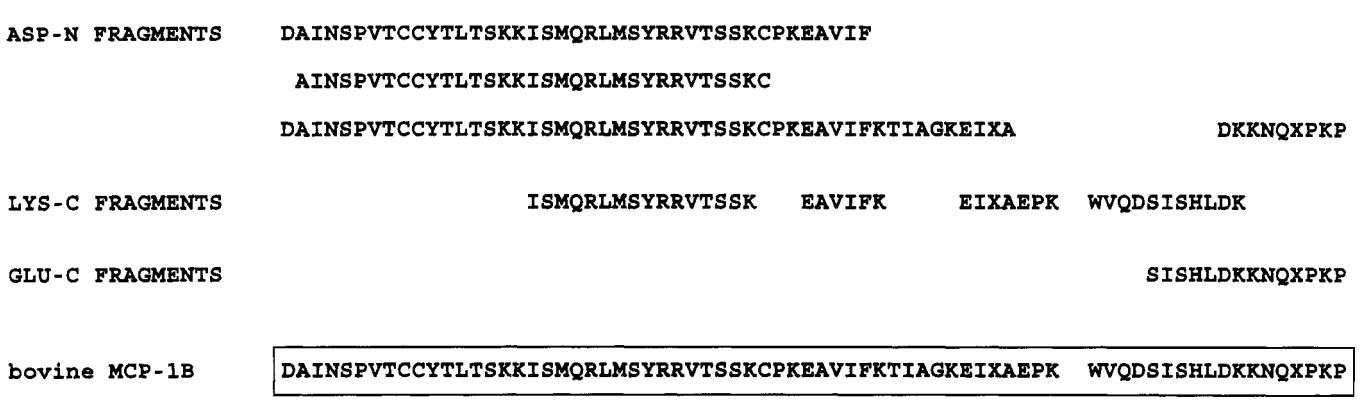

FIGURE 6: Amino acid sequence alignment of proteolytic fragments of natural bo MCP-1B obtained by endoproteinase Asp-N, Lys-C, and Glu-C digestion. Unidentified residues are marked as X.

Table 1: Identity (%) between Human (hu), Murine (mu), and Bovine (bo) MCPs

	bo MCP-1B	bo MCP-1	bo MCP-2	hu MCP-1	hu MCP-2	hu MCP-3	mu JE	mu MARC
bo MCP-1B	100	84	53	77	57	64	58	57
bo MCP-1	84	100	51	72	51	64	54	54
bo MCP-2	53	51	100	55	67	57	54	57
hu MCP-1	77	72	55	100	62	71	55	57
hu MCP-2	57	51	67	62	100	58	53	50
hu MCP-3	64	64	57	71	58	100	51	59
mu JE	58	54	54	55	53	51	100	51
mu MARC	57	54	57	57	50	59	51	100

(data not shown). FPLC fractions were also tested for monocyte chemotactic activity using the microchamber migration assay. The main monocyte chemotactic activity was found to elute at about 0.5 M NaCl (fractions 33−38), corresponding to the first peak of β-glucuronidase-releasing activity.

For further purification and biological characterization of proteins active on monocytes, RP-HPLC on a narrow-bore C-8 column was used. Bovine monocyte chemotactic activity recovered from FPLC at 0.5 M NaCl (fractions 34−36) was found to elute at an acetonitrile concentration of 24%. At this position (fraction 35) a 14-kDa protein (25 μg/mL) was detectable by SDS−PAGE (Figure 4). The minimal effective concentration (0.6 nM) of this bovine monocyte chemotactic protein (MCP) was approximately 3 times higher than the amount needed for human MCP-1 (0.17 nM) when tested in the microchamber assay using human monocytes (Figure 5A). The maximal chemotactic response of both bovine MCP and human MCP-1 was reached at 1.8 nM. Both proteins showed a typical bell-shaped dose−response curve similar to those obtained for most chemotactic factors. In analogy to human MCP-1, this bo MCP was unable to attract human neutrophil granulocytes (Figure 5B) and eosinophils (data not shown).

Identification of the Bovine Chemotactic Protein by Amino Acid Sequence Analysis. A first attempt to identify the purified bovine MCP by NH_2-terminal sequence analysis was not successful. Since the NH_2 terminus of this 14-kDa protein appeared to be blocked for Edman degradation, internal proteolytic fragments of pure protein were generated by proteolysis with endoproteinase Asp-N, Lys-C, or Glu-C. Fragments were purified by C-8 RP-HPLC, and sequence analysis yielded multiple unique sequences. Alignment of these peptide sequences (Figure 6) revealed nearly the complete amino acid sequence of a novel protein not present in the Swiss-prot protein data bank (April 1994). The primary structure of the bovine monocyte chemotactic protein was characterized by four cysteines typical for the chemokine family of chemotactic cytokines, including the human monocyte chemotactic proteins MCP-1, MCP-2, and MCP-3 (Van Damme et al., 1992).

When compared to bovine MCP-1 (Wempe et al., 1991) and MCP-2 (Wempe et al., 1994), the novel bovine protein contains 84% and 53% identical residues, respectively (Table 1; Figure 7). Its amino acid sequence was also more related to that of human MCP-1 (77% identity) than to those of human MCP-2 and MCP-3 (57% and 64% identity, respectively). Bovine MCP-1, however, also shows higher similarity to human MCP-1 (72%) than to human MCP-2 (51%) and MCP-3 (64%). We therefore decided to consider the novel protein as an additional bovine monocyte chemotactic protein-1, designated bo MCP-1B (Table 1; Figure 7).

In view of its similarity to other MCPs, bovine MCP-1B is expected to contain two additional NH_2-terminal residues (including pyroglutamate). Therefore, intact protein and blocked NH_2-terminal fragments (from a RP-HPLC-purified endoproteinase Lys-C digest) were treated with pyroglutamate aminopeptidase and repurified on C-8 RP-HPLC. However, no further sequence information could be obtained after Edman degradation.

DISCUSSION

The discovery of novel chemotactic cytokines that attract specific types of leukocytes has improved our understanding of selective cell migration during inflammation, infection, tumor invasion, or immune diseases (Mantovani et al., 1992; Opdenakker & Van Damme, 1994). The fact that chemokines of various animal species have been independently identified from different cellular sources illustrates the wide interest in the biological significance of these molecules [reviewed in Oppenheim et al. (1991), Van Damme (1994), and Schall (1991)]. Since the isolation of human IL-8, other structurally related members of the C-X-C chemokine

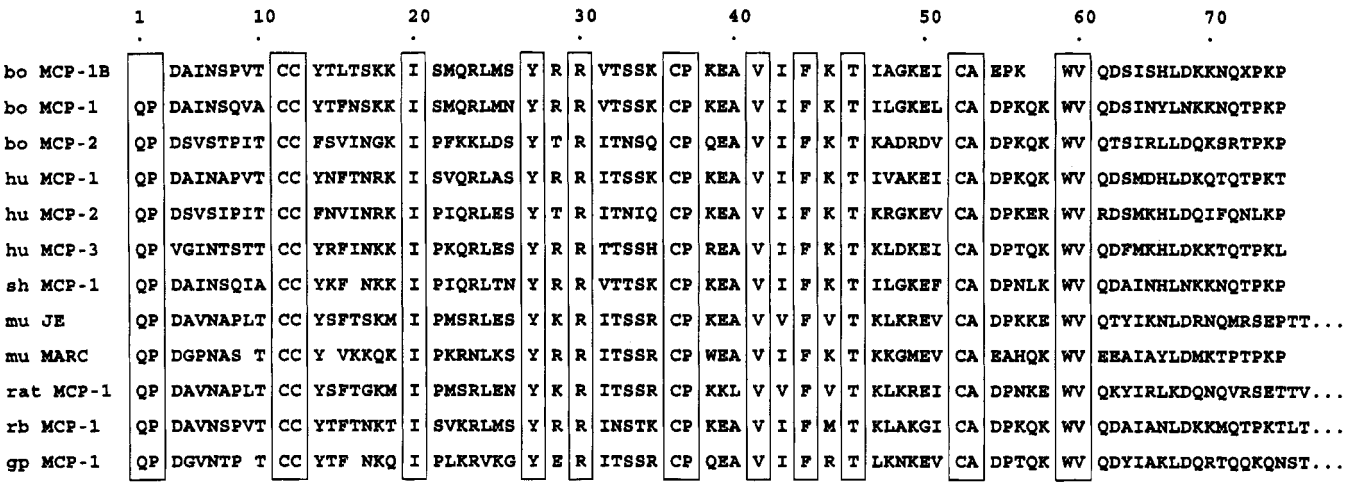

FIGURE 7: Amino acid sequence alignment of bovine (bo) MCP-1B with other MCP molecules of the C-C chemokine family: bo MCP-1 (Wempe et al., 1991), bo MCP-2 (Wempe et al., 1994), human (hu) MCP-1 (Yoshimura et al., 1989; Furutani et al., 1989; Robinson et al., 1989), hu MCP-2 (Schall, 1991; Van Damme et al., 1992), hu MCP-3 (Opdenakker et al., 1993), sheep (sh) MCP-1 (Yoshimura, 1993), murine (mu) JE (Rollins et al., 1988), mu MARC (Kulmburg et al., 1992;Thirion et al., 1994), rat MCP-1 (Yoshimura et al., 1991), rabbit (rb) MCP-1 (Yoshimura & Yukhi, 1991), and guinea pig (gp) MCP-1 (Yoshimura, 1993). Identical residues in all sequences are boxed.

subfamily have been discovered, but the counterpart of IL-8 in most other animal species, e.g., mouse, has not yet been isolated. In contrast, for the youngest C-X-C chemokine member, granulocyte chemotactic protein 2 (GCP-2; Proost et al., 1993a,b) both the human and bovine proteins are identified.

In the bovine system (Wempe et al., 1991, 1994) two possible homologues for the human C-C chemokines MCP-1, MCP-2, and MCP-3 (Van Damme et al., 1992) have been identified. Bovine MCP-1 was originally isolated from seminal vesicle secretary epithelium, whereas bovine MCP-2 was found to be expressed in peripheral mononuclear leukocytes. We have now isolated a third monocyte chemotactic protein from cultured bovine kidney cells, designated bo MCP-1B on the basis of its high structural similarity to bovine MCP-1 (84% identity). Although MDBK cells spontaneously secrete monocyte chemotactic activity, increased production was observed after PMA treatment.

Similar to most chemokines, including bovine GCP-2 (Proost et al., 1993b), bovine MCP-1B could be purified from conditioned medium by its affinity for heparin. Monocyte and granulocyte chemotactic activities from MDBK cells were subsequently fractionated by cation-exchange FPLC. Finally C-8 RP-HPLC allowed purification of MCP-1B to homogeneity. As reported for other MCPs, bovine MCP-1B was found to be NH_2-terminally blocked. Sequence analysis of multiple proteolytic fragments resulted in the identification of nearly the complete primary structure of bovine MCP-1B. The amino acid sequence of bovine MCP-1B was found to be unique and resembled best those of bovine and human MCP-1. Since bovine MCP-1 is also highly related (72% identity) to human MCP-1 (Wempe et al., 1991) it is difficult to conclude which one of the two bovine MCPs is the real counterpart for human MCP-1. In addition, bovine MCP-1 and MCP-1B show an equal but lower structural similarity (64% identity) to human MCP-3. Human MCP-3 has been isolated from cytokine-stimulated osteosarcoma cells (Van Damme et al., 1992; Opdenakker et al., 1993) but also from PMA-induced promonocytic cells (Minty et al., 1993).

The regulation of bovine MCP-1 expression is at present poorly documented. Cellular sources and inducers of human and mouse MCP-1 have already been studied in more detail (Oppenheim et al., 1991). Multiple cell types can produce MCP-1 and MCP-2 if appropriately stimulated with cytokines or viral or bacterial products (Van Damme et al., 1989; Van Damme, 1994). Although human MCP-1, MCP-2, and MCP-3 have a comparable specific activity on monocytes, they attract basophils and eosinophils to different extents (Alam et al., 1994; Dahinden et al., 1994; Noso et al., 1994). In contrast to human MCP-3, but like human MCP-1, the herein-described bovine MCP-1B is not chemotactic for human eosinophils, indicating that biologically the latter two might be more related (results not shown). A further study of the regulated secretion by various producer cell types and of possible other target cells (e.g., basophils, lymphocytes) could allow definition of which human MCP is the real counterpart for bovine MCP-1B. Recently, it has been shown that human MCP-1 and MCP-2 production are differently regulated (Van Damme et al., 1994) and that these chemokines use a different signaling pathway on monocytes (Sozzani et al., 1994).

The identification of the various chemokines in different animal species should help us to understand the apparent redundancy in the chemokine family. For each species a particular set of chemokines might determine the inflammatory response either by means of the relative secretion level or by the selective biological effect on individual leukocyte subtypes. Further characterization of the individual chemokines should allow determination of their specific roles in various pathologies.

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