Biochemical Pharmacology, Vol. 59, pp. 591–596, 2000.

ISSN 0006-2952/00/$–see front matter
PII S0006-2952(99)00354-8

Selective Binding of the Truncated Form of the Chemokine CKβ8 (25–99) to CC Chemokine Receptor 1 (CCR1)

Theo A. Berkhout,† Jayneeta Gohil,* Pilar Gonzalez,* Charlotte L. Nicols,* Kitty E. Moores,* Colin H. Macphee,* John R. White‡ and Pieter H.E. Groot**

*Vascular Biology, SmithKline Beecham Pharmaceuticals, Harlow CM19 5AD, U.K.; and ‡Molecular and Cellular Immunology, SmithKline Beecham, PO Box 1539, King of Prussia, PA 19406, U.S.A.

ABSTRACT. Human CC chemokine receptor 1 (CCR1) has been proposed as a receptor for CKβ8. To obtain conclusive evidence, binding–displacement studies of ^{125}I-CKβ8 (25–99) were performed on membranes of Chinese hamster ovary cells expressing human CCR1. The IC_{50} for displacement of ^{125}I-CKβ8 (25–99) with CKβ8 (25–99) was 0.22 nM. The longer forms of CKβ8 (24–99 and 1–99) also displaced ^{125}I-CKβ8, with IC_{50} values of 6.5 and 16 nM, respectively. Displacement profiles of ^{125}I-CKβ8 (25–99) on freshly prepared human monocytes indicated that CCR1 was the major receptor for CKβ8. We conclude that CCR1 is a receptor for different-length CKβ8 and that CKβ8 (25–99) has a similar affinity for CCR1 as macrophage inflammatory protein-1α (MIP-1α). The longer variants of CKβ8 are significantly less potent than CKβ8 (25–99) and MIP-1α on CCR1 and monocytes ($P < 0.05$). BIOCHEM PHARMACOL **59**;5:591–596, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. CKβ8; human CC chemokine receptor 1 (CCR1); monocytes; ligand binding; chemokine; chemokine receptor

Chemokines are small basic proteins that play an important role in chemotaxis and activation of leukocytes [1]. Based on the positional arrangement of the cysteines, four families of chemokines have been identified: CXC, CC, CXXXC, and C. The CXC chemokines mainly attract and activate neutrophils. Five receptors for these chemokines have been identified, one of which (CXCR5) was matched to B cell-attracting chemokine 1 (BCA-1) quite recently [2].

The second group, the CC chemokines, mainly attracts monocytes and activated T cells, but some also attract eosinophils and basophils. Ten receptors for the CC chemokines have been identified (CCR1§ to CCR10). Lymphotactin [3, 4] is the only known representative of a C chemokine and acts on T lymphocytes. Its receptor was identified recently as GPR-5 (XCR1) [5]. Fractalkine or neurotactin represents the fourth class of CXXXC chemokines [6, 7]. Its receptor has now been identified as the rat orphan receptor RBS-11 [8].

CKβ8, also known as myeloid progenitor inhibitory factor-1 (MPIF-1) or macrophage inflammatory protein-3 (MIP-3), is a member of the CC family that has been identified via the expressed sequence tag sequencing effort by Human Genome Sciences [9]. Recently, it was shown that this chemokine has the highest homology with leukotactin-1 (Lkn-1) [10]. A longer splice variant of CKβ8 exists, known as CKβ8-1 [11]. CKβ8 was mapped to chromosome 17q just 200 kb separated from Lkn-1 [11] and close to another novel chemokine Lymphocyte and Monocyte Chemoattractant (LMC) [12]. CKβ8 mRNA is highly expressed in pancreas and skeletal muscle [11]. Initially, two groups published data on functional responses with the 99-amino-acid form of this chemokine [9, 13]. In these papers, weak functional responses with monocytes were observed, but a substantial chemotactic response in resting T lymphocytes [9] was not confirmed by the second group [13]. We have recently described two shorter forms of CKβ8 that are more potent than the long form, containing amino acids 24–99 and 25–99 of the secreted chemokine [14]. The very potent short form of CKβ8 (25–99) evoked a chemotactic response in monocytes with an EC_{50} of 54 pM, as compared to 80 nM for the long form. Cross-desensitisation experiments suggested that CCR1 was the predominant receptor that mediates the calcium response. Incomplete desensitisation suggested that there were additional receptors involved. To further define the receptor usage, we measured binding of ^{125}I-CKβ8 (25–99) and ^{125}I-MIP-1α to membranes of a CCR1-expressing cell line as well as freshly isolated monocytes.

† Corresponding author: Dr. Theo A. Berkhout, SmithKline Beecham Pharmaceuticals, NFSP(N), H30-1-50, Coldharbour Road, Harlow CM19 5AD, U.K. Tel. 44 1279 627040; FAX 44 1279 627049; E-mail: Theo_Berkhout-1@sbphrd.com

§ *Abbreviations:* CCR1, human CC chemokine receptor 1; CHO, Chinese hamster ovary; MCP-3, monocyte chemotactic protein-3; MIP-1, macrophage inflammatory protein-1; and RANTES, regulated on activation, normal T cell expressed and secreted.

Received 19 January 1999; accepted 3 August 1999.

MATERIALS AND METHODS

Materials

^{125}I-RANTES and ^{125}I-MIP-1α were from Amersham. ^{125}I-CKβ8 (25–99) was custom-labelled by Amersham using enzymatic iodination with sodium [^{125}I] iodide, hydrogen peroxide, and lactoperoxidase to a specific activity of 2000 μCi/μmol. Unlabelled chemokines were purchased from R&D Systems and Peprotech. Albumin fraction V, fatty acid-free, was from Roche Diagnostics. CCR1 membranes produced in CHO cells were from BioSignal Inc. CKβ8 (24–99) and (25–99) were postexpression species of CKβ8 (1–99) and were purified as described elsewhere [14]. Human peripheral monocytes were prepared from the blood of normal healthy volunteers as described elsewhere [15].

Chemokine Receptor Membrane Binding Assays

Binding of ^{125}I-MIP-1α and ^{125}I-CKβ8 (25–99) to CCR1 membranes was carried out according to the protocol (BioSignal) in a HEPES buffer at 27° for 60 min. Binding of ^{125}I-CKβ8 (25–99) to monocytes was as described elsewhere [15]. Briefly, 0.5–1 × 10^6 cells were incubated in a 96 deep well polystyrene plate with 75,000 dpm ^{125}I-CKβ8 (25–99) or ^{125}I-MIP-1α at 37° for 15 min in RPMI-1640 medium (without bicarbonate)/0.2% BSA/0.1% azide pH 7.0.

RESULTS

Binding of CKβ8 to CCR1

Some previous functional data suggested that CCR1 was a receptor for CKβ8 [11, 14]. We aimed to obtain direct proof for this. The short form of CKβ8 (25–99) was used as a suitable radioligand because this was found to be the most potent of the CKβ8 forms in recent functional studies on monocytes and eosinophils [14]. Figure 1A shows binding of the CCR1 ligand ^{125}I-MIP-1α to CCR1 membranes and its displacement by CKβ8 (25–99), MIP-1α, RANTES, and MCP-3. The short form of CKβ8 (25–99) is the most potent chemokine to displace ^{125}I-MIP-1α, with an IC_{50} of 33 pM (SD ± 40 pM, N = 5). MIP-1α displaced ^{125}I-MIP-1α with an IC_{50} of 0.14 nM (SD ± 0.11 nM, N = 4), which was significantly higher than CKβ8 (25–99) in a *t*-test (one-tail, $P < 0.05$). MCP-3 displaced ^{125}I-MIP-1α with an IC_{50} of 1.6 nM (SD ± 1.2 nM, N = 3), whereas RANTES only partially displaced ^{125}I-MIP-1α. The data suggest that CKβ8 (25–99) binds strongly to the CCR1 receptor with an affinity somewhat higher than that of MIP-1α. To confirm this, we used custom-labelled ^{125}I-CKβ8 (25–99). Figure 1B shows that ^{125}I-CKβ8 (25–99) binds with high affinity to CCR1. A K_d of 0.16 nM (SD ± 0.035, N = 4) was obtained which was not significantly different from that of ^{125}I-MIP-1α in a *t*-test (not shown, K_d 0.20 nM, SD ± 0.017, N = 3). The B_{max} value was 0.6 fmol/μg protein for both radiolabelled chemokines. This is in agreement with a one-to-one binding ratio of the ligand to the CCR1 receptor.

The IC_{50} for the homologous displacement of ^{125}I-CKβ8 (25–99) from CCR1 with CKβ8 (25–99) was 0.22 nM (SD ± 0.09 nM, Fig. 1C). The IC_{50} value for MIP-1α was 0.66 nM (SD ± 0.48 nM), which was significantly higher than for CKβ8 (25–99) ($P < 0.02$, *t*-test). MCP-3 was a weak antagonist, with an IC_{50} of 11 nM (SD ± 13 nM). The observed partial inhibition with RANTES might have been caused by aggregation and dimerisation, phenomena observed with this chemokine by other authors [16]. A partial displacement of ^{125}I-CKβ8 (25–99) was also repeatedly observed with MIP-1α. The affinity of the different forms of CKβ8 for CCR1 is compared in Fig. 2. CKβ8 (24–99) displaced ^{125}I-CKβ8 (25–99) with an IC_{50} of 7 nM (SD ± 6 nM), whereas for CKβ8 (1–99) the IC_{50} was 16 nM, SD ± 13 nM. The decrease in potency as compared to CKβ8 (25–99) was highly significant for the 24–99 ($P < 0.005$, *t*-test) and the 1–99 ($P < 0.002$, *t*-test). We also measured the binding competition of ^{125}I-CKβ8 (25–99) and ^{125}I-RANTES to CHO cell membranes expressing CCR5. Radiolabelled CKβ8 (25–99) did not bind to CCR5 and radiolabelled RANTES was not displaced by CKβ8 (25–99) (not shown).

Binding of CKβ8 to Monocytes

To investigate binding to cells previously shown to give functional responses with CKβ8, we tested binding of ^{125}I-CKβ8 (25–99) to monocytes. ^{125}I-CKβ8 (25–99) bound with high affinity to freshly isolated peripheral blood monocytes, with a K_d of 0.31 nM (N = 3, SD ± 0.18 nM) (Fig. 3A). This was not significantly different from the binding of ^{125}I-MIP-1α to monocytes (not shown, N = 3, K_d = 0.49 ± 0.12 nM). From the B_{max}, the receptor number/cell was calculated to be around 17,000 for both radiolabels. Homologous displacement of ^{125}I-CKβ8 was achieved with an IC_{50} of 0.51 nM (SD ± 0.34 nM, Fig. 3B), whereas MIP-1α competed with an IC_{50} of 0.62 nM (SD ± 0.37 nM). These results were very similar to those obtained for the displacement of ^{125}I-MIP-1α (Fig. 3C). In these experiments, the displacement of ^{125}I-MIP-1α by MIP-1α seemed a factor 2–3 times more efficient than the displacement by CKβ8 (25–99), although this did not reach significance in a *t*-test. The IC_{50}s for displacement of MIP-1α and CKβ8 (25–99) were 0.41 ± 0.24 nM and 1.0 ± 0.9 nM, respectively.

Table 1 compares the different potencies of the various forms of CKβ8 with our previously published functional data [14]. The first conclusion is that the binding potencies generally match well with the elicited functional responses. CKβ8 (25–99) displaces ^{125}I-CKβ8 (25–99) with about 100 times more potency than CKβ8 (1–99), whereas for chemotaxis the difference in potency is about 1000-fold. Generally, the IC_{50}s of MIP-1α and the different forms of CKβ8 appeared somewhat higher on fresh monocytes than on CCR1 membranes. This difference only reached significance for CKβ8 (25–99) ($P < 0.05$) for both radioligands.

Monocytes are thought to be the main target cells for

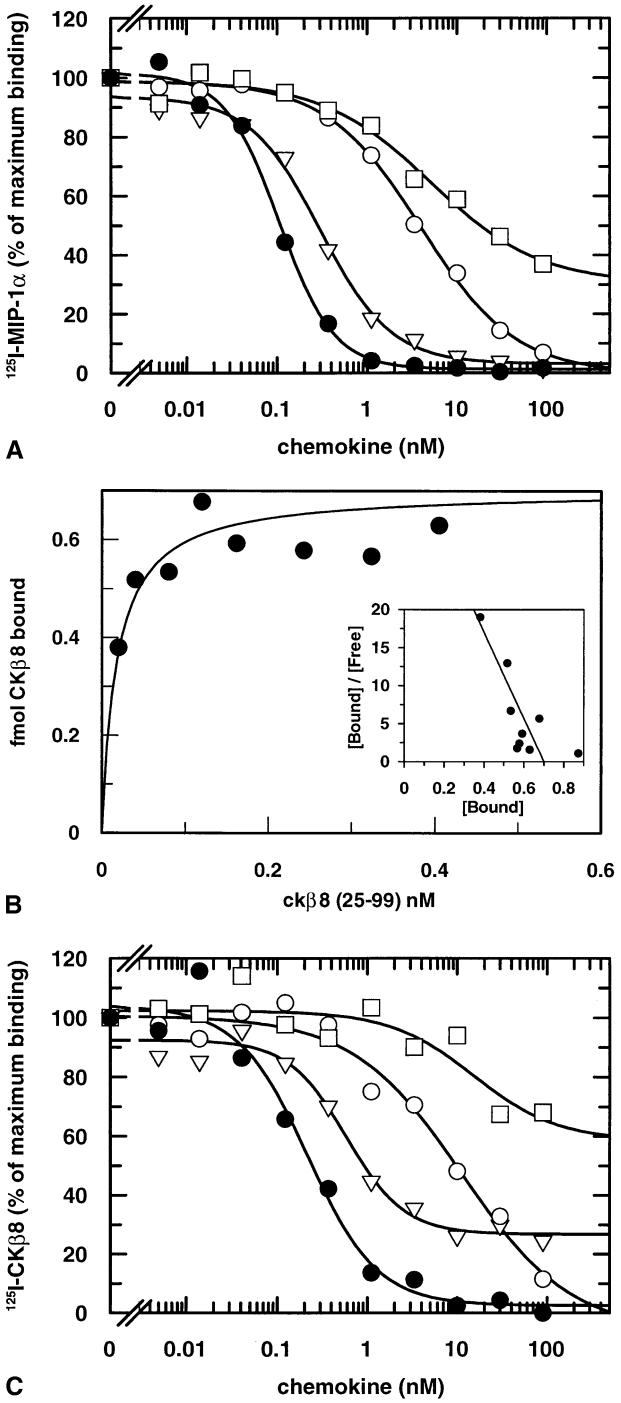

FIG. 1. Binding–displacement of ^{125}I-MIP-1α and ^{125}I-CKβ8 (25–99) on CCR1 membranes. Membranes from CHO cells expressing CCR1 were incubated with 75,000 dpm ^{125}I-MIP-1α (A) in the presence of MIP-1α (▽), CKβ8 (25–99; ●), MCP-3 (○), and RANTES (□) for 1 hr at 27°. (B) Specific binding of ^{125}I-CKβ8 (25–99) to CCR1 membranes and Scatchard analysis. For non-specific binding, 100 nM CKβ8 (25–99) was included at each concentration and the non-specific binding was subtracted from the total binding to give specific binding. (C) Membranes from CHO cells expressing CCR1 were incubated with 75,000 dpm ^{125}I-CKβ8 (25–99) as described under (A) above. The 100% value represents approximately 6000 dpm specific binding for both radiolabelled MIP-1α and CKβ8 (25–99). The experiments were repeated at least twice with similar results.

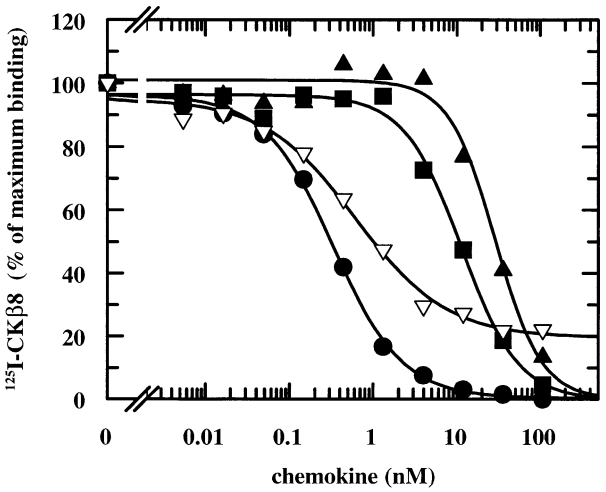

FIG. 2. Binding–displacement of ^{125}I-CKβ8 (25–99) from CCR1 membranes by different CKβ8 forms. Membranes from CHO cells expressing CCR1 were incubated with radiolabelled CKβ8 (25–99; 75,000 dpm) in the presence of CKβ8 (25–99; ●), CKβ8 (24–99; ■), CKβ8 (1–99; ▲), and MIP-1α (▽), as described in Fig. 1. This experiment was repeated three times with similar results.

CKβ8 [13], and functional and chemotaxis data suggested the existence of more than one type of chemokine receptor for CKβ8 in this cell. We investigated this in binding studies on fresh human monocytes using a panel of 52 chemokines to displace ^{125}I-CKβ8 (25–99). Only the different forms of CKβ8, MIP-1α, MCP-3, and RANTES were able to displace ^{125}I-CKβ8 (not shown). These results are consistent with CCR1 being the sole binding site for CKβ8 (25–99) on monocytes.

DISCUSSION

Our binding studies show unequivocally that CKβ8 (1–99), but also the CKβ8 (24–99) and CKβ8 (25–99) variants, bind to CCR1, with the latter (the shortest variant) binding with high affinity. The IC_{50} of CKβ8 (25–99) was significantly lower than that of MIP-1α in homologous displacement, but not with ^{125}I-MIP-1α (see Table 1). As the K_ds of both ^{125}I-CKβ8 (25–99) and ^{125}I-MIP-1α were not significantly different on either monocytes or CCR1 membranes, one must be prudent in concluding which chemokine is more potent. Although desensitisation studies have suggested that CCR1 may be the receptor for CKβ8, binding of this ligand and its different-length variants has not been documented [9, 13, 14].

From the arguments stated below, we conclude that the response of monocytes to CKβ8 (25–99) is fully explained by the interaction with CCR1. Firstly, a very recent report showed that two splice variants of CKβ8, one of which represents CKβ8 (1–99), elicited calcium transients in HOS cells transfected with CCR1, but not in cells transfected with CCR2B, CCR3, CCR4, CCR5, and CXCR4 [11]. In agreement with this, we have observed no binding

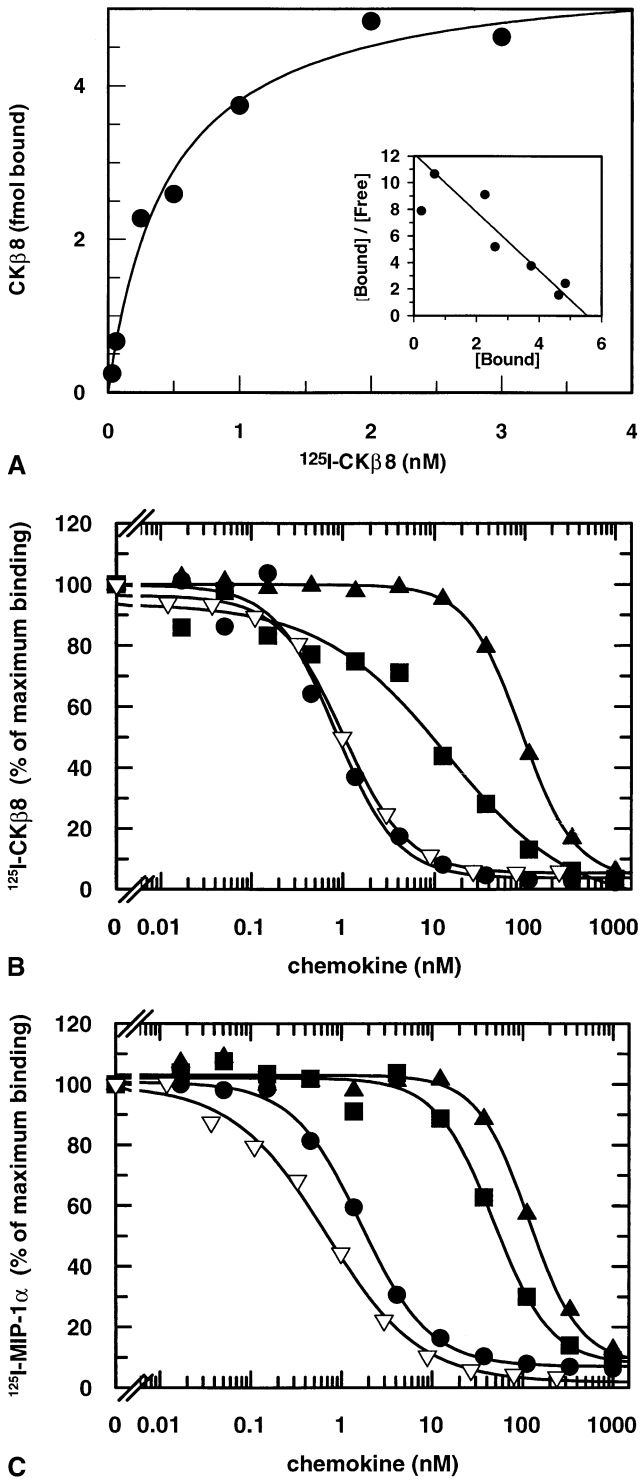

FIG. 3. Binding–displacement of ^{125}I-CKβ8 and ^{125}I-MIP-1α on fresh human monocytes. (A) Monocytes were incubated with different concentrations of ^{125}I-CKβ8 (25–99) for 15 min at 37°. For non-specific binding, 100 nM CKβ8 (25–99) was included. (B) Displacement on fresh human monocytes of ^{125}I-CKβ8 (25–99) or (C) ^{125}I-MIP-1α with CKβ8 (25–99; ●), CKβ8 (24–99; ■), CKβ8 (1–99; ▲), and MIP-1α (▽). Monocytes were incubated for 15 min at 37° in the presence of 0.1% azide. Maximum binding (100%) represents about 10,000 dpm for both ^{125}I-CKβ8 (25–99) and ^{125}I-MIP-1α. The experiments were repeated at least twice with similar results.

of CKβ8 (25–99) to CCR2B, CCR3, CCR5, and CCR8, and no functional interactions of CKβ8 variants with CCR3, CCR6, and CCR7 in expressing cell lines or membranes (not shown). Secondly, the binding–displacement profile of ^{125}I-CKβ8 (25–99) with the different CKβ8 forms on monocytes reflects the profile obtained for CCR1 membranes. Thirdly, of a panel of 52 chemokines only those known to be recognised by CCR1 could displace ^{125}I-CKβ8 (25–99) from monocytes.

This finding is somewhat in contrast with our previous functional studies showing incomplete desensitisation of CKβ8 (25–99)-elicited calcium transients by RANTES, but a full desensitisation of RANTES-induced calcium transients by CKβ8 on monocytes [14]. Our previous interpretation of these results was that CKβ8 might bind to the RANTES receptor CCR5 or to a novel CKβ8 receptor. With binding studies, one has to be aware that only homologous binding assays may be a true indication of affinity to the receptor, as was shown for the tachykinin receptors [17]. We therefore tested the potential involvement of CKβ8 in binding to CCR5 in homologous binding studies with ^{125}I-CKβ8 (25–99) rather than using only ^{125}I-RANTES. However, we observed no binding of ^{125}I-CKβ8 to CCR5, which eliminates CCR5 as a CKβ8 (25–99) receptor. Our binding studies, however, do not preclude the existence of a minor population of lower-affinity CKβ8 receptors, which might also explain the desensitisation results. On the other hand, the partial displacement of ^{125}I-CKβ8 (25–99) by MIP-1α and RANTES on CCR1 membranes (Figs. 1 and 2) suggests that part of CKβ8 (25–99) is bound with high affinity to a specific conformation of CCR1, where it cannot be displaced by MIP-1α and RANTES. For some reason, we do not observe this displacement pattern in monocytes, but it has been observed occasionally in THP-1 cells. The assumption that even a minor population of CCR1 binds CKβ8 but does not bind RANTES might explain how RANTES cannot fully desensitise CCR1-mediated calcium-elicited transients by CKβ8 (25–99). However, the K_d results for ^{125}I-MIP-1α and ^{125}I-CKβ8 (25–99) on CCR1 suggest a similar B_{max}, which does not support the hypothesis of a CCR1 form that solely binds CKβ8 and not MIP-1α.

The truncation of just one amino acid from CKβ8 (24–99) results in a huge increase in the IC_{50} in binding–displacement studies on the CCR1 receptor. It is well documented that a small change in the amino terminal of chemokines can greatly affect their binding or functional properties [18, 19]. When one compares the sequences of CKβ8 (1–99) with its splice variant CKβ8-1 [11], macrophage inflammatory protein-3, and leukotactin-1, one can observe that despite the great homology, the aspartic acid residue in the 24-position is specific to the CKβ8 (1–99) form. This aspartic acid residue might be crucial for the generation of the very potent CKβ8 (25–99) form via proteolytic cleavage. One can speculate that any differences in tissue expression between CKβ8 (1–99) and CKβ8-1

TABLE 1. Comparison of binding–displacement and functional responses of different-length CKβ8 on fresh human peripheral monocytes and CCR1

	MIP-1α	CKβ8 (25–99)	CKβ8 (24–99)	CKβ8 (1–99)
Ca^{2+} transients	0.27 ± 0.03 nM	0.38 ± 0.04 nM	11 nM	150 ± 19 nM
Chemotaxis	0.25 ± 0.06 nM	0.054 ± 0.005 nM	ND	80 ± 7 nM
Binding to monocytes	0.62 ± 0.37 nM	0.51 ± 0.34 nM	11 ± 3.2 nM	37 ± 36 nM
	N = 5	N = 9	N = 4	N = 4
Binding to CCR1	0.66 ± 0.48 nM	0.22 ± 0.14 nM	7 ± 6 nM	16 ± 13 nM
	N = 7	N = 9	N = 4	N = 4

Data from the calcium transients and chemotaxis were recently published [14] and expressed as EC_{50}s ± SD. The displacement data are presented as the means of the IC_{50} ± SD and the number of experiments. For binding competition with ^{125}I-CKβ8 (25–99) on monocytes, the IC_{50} of each chemokine tested significantly different from the other chemokines (*t*-test, two-tail, probability at least <0.05) except for the pair of CKβ8 (1–99) with CKβ8 (24–99). The IC_{50} of MIP-1α was significantly higher than CKβ8 (25–99) in a one-tail *t*-test only ($P < 0.05$). The statistics on CCR1 were very similar, except that there was a significant difference observed (two-tail, $P < 0.02$) between MIP-1α and CKβ8 (25–99). However, this statistical difference was not seen when ^{125}I-MIP-1α was used as the radioligand on either monocytes or CCR1 membranes (not shown). ND, not determined.

[11] and possibly other splice variants as well [20] provide an efficient mechanism for tissue-specific release of the highly potent CKβ8 (25–99) variant via proteolytic cleavage. More studies are required to establish the physiological existence of CKβ8 (25–99) and its role in health and disease.

We would like to acknowledge the technical contributions by the sandwich students Tom Molenaar and Suzanne Hendriksen. We express thanks to our SB colleagues Ed Appelbaum and Skip Sarau for their helpful discussions regarding the effects of CKβ8 (25–99) on other CCR-transfected cell lines as mentioned in the discussion section of this paper.

References

1. Baggiolini M, Chemokines and leukocyte traffic. *Nature* **392:** 565–568, 1998.
2. Legler DF, Loetscher M, Roos RS, Clark-Lewis I, Baggiolini M and Moser B, B cell-attracting chemokine 1, a human CXC chemokine expressed in lymphoid tissues, selectively attracts B lymphocytes via BLR1/CXCR5. *J Exp Med* **187:** 655–660, 1998.
3. Kelner GS, Kennedy J, Bacon KB, Kleyensteuber S, Largaespada DA, Jenkins NA, Copeland NG, Bazan JF, Moore KW and Schall TJ, Lymphotactin: A cytokine that represents a new class of chemokine. *Science* **266:** 1395–1399, 1994.
4. Kennedy J, Kelner GS, Kleyensteuber S, Schall TJ, Weiss MC, Yssel H, Schneider PV, Cocks BG, Bacon KB and Zlotnik A, Molecular cloning and functional characterization of human lymphotactin. *J Immunol* **155:** 203–209, 1995.
5. Yoshida T, Imai T, Kakizaki M, Nishimura M, Takagi S and Yoshie O, Identification of single C motif-1/lymphotactin receptor XCR1. *J Biol Chem* **273:** 16551–16554, 1998.
6. Pan Y, Lloyd C, Zhou H, Dolich S, Deeds J, Gonzalo JA, Vath J, Gosselin M, Ma JY, Dussault B, Woolf E, Alperin G, Culpepper J, Gutierrezramos JC and Gearing D, Neurotactin, a membrane-anchored chemokine up-regulated in brain inflammation. *Nature* **387:** 611–617, 1997.
7. Schall T, Fractalkine—A strange attractor in the chemokine landscape. *Immunol Today* **18:** 147, 1997.
8. Imai T, Hieshima K, Haskell C, Baba M, Nagira M, Nishimura M, Kakizaki M, Takagi S, Nomiyama H, Schall TJ and Yoshie O, Identification and molecular characterization of fractalkine receptor CX_3CR1, which mediates both leukocyte migration and adhesion. *Cell* **91:** 521–530, 1997.
9. Patel VP, Kreider BL, Li YL, Li HD, Leung K, Salcedo T, Nardelli B, Pippalla V, Gentz S, Thotakura R, Parmelee D, Gentz R and Garotta G, Molecular and functional characterization of 2 novel human C-C chemokines as inhibitors of 2 distinct classes of myeloid progenitors. *J Exp Med* **185:** 1163–1172, 1997.
10. Youn BS, Zhang SM, Lee EK, Park DH, Broxmeyer HE, Murphy PM, Locati M, Pease JE, Kim KK, Antol K and Kwon BS, Molecular cloning of leukotactin-1: A novel human beta-chemokine, a chemoattractant for neutrophils, monocytes, and lymphocytes, and a potent agonist at CC chemokine receptors 1 and 3. *J Immunol* **159:** 5201–5205, 1997.
11. Youn BS, Zhang SM, Broxmeyer HE, Cooper S, Antol K, Fraser M and Kwon BS, Characterization of CKbeta8 and CKbeta8–1: Two alternatively spliced forms of human beta-chemokine, chemoattractants for neutrophils, monocytes, and lymphocytes, and potent agonists at CC chemokine receptor 1. *Blood* **91:** 3118–3126, 1998.
12. Youn BS, Zhang S, Broxmeyer HE, Antol K, Fraser MJ Jr, Hangoc G and Kwon BS, Isolation and characterization of LMC, a novel lymphocyte and monocyte chemoattractant human CC chemokine, with myelosuppressive activity. *Biochem Biophys Res Commun* **247:** 217–222, 1998.
13. Forssmann U, Delgado MB, Uguccioni M, Loetscher P, Garotta G and Baggiolini M, Ckbeta8, a novel CC chemokine that predominantly acts on monocytes. *FEBS Lett* **408:** 211–216, 1997.
14. Macphee CH, Appelbaum ER, Johanson K, Moores KE, Imburgia CS, Fornwald J, Berkhout T, Brawner M, Groot PE, O'Donnell K, O'Shannessy D, Scott G and White JR, Identification of a truncated form of the CC chemokine ck-beta-8 demonstrating greatly enhanced biological activity. *J Immunol* **161:** 6273–6279, 1998.
15. Berkhout TA, Sarau HM, Moores K, White JR, Elshourbagy N, Appelbaum E, Reape RJ, Brawner M, Makwana J, Foley JJ, Schmidt DB, Imburgia C, McNulty D, Matthews J, O'Donnell K, O'Shannessy D, Scott M, Groot PE and Macphee C, Cloning, *in vitro* expression, and functional characterization of a novel human CC chemokine of the monocyte chemotactic protein (MCP) family (MCP-4) that binds and signals through the CC chemokine receptor 2B. *J Biol Chem* **272:** 16404–16413, 1997.
16. Wang JM, McVicar DW, Oppenheim JJ and Kelvin DJ, Identification of RANTES receptors on human monocytic cells: Competition for binding and desensitization by homologous chemotactic cytokines. *J Exp Med* **177:** 699–705, 1993.
17. Hastrup H and Schwartz TW, Septide and neurokinin A are high-affinity ligands on the NK-1 receptor: Evidence from

homologous versus heterologous binding analysis. *FEBS Lett* **399:** 264–266, 1996.
18. Proudfoot AE, Power CA, Hoogewerf A, Montjovent MO, Borlat F and Wells TN, Characterisation of the RANTES/MIP-1 alpha receptor (CC CKR-1) stably transfected in HEK 293 cells and the recombinant ligands. *FEBS Lett* **376:** 19–23, 1995.
19. Keane MP, Arenberg DA, Moore BB, Addison CL and Strieter RM, CXC chemokines and angiogenesis/angiostasis. *Proc Assoc Am Physicians* **110:** 288–296, 1998.
20. Wells TN and Peitsch MC, The chemokine information source: Identification and characterization of novel chemokines using the WorldWideWeb and expressed sequence tag databases. *J Leukoc Biol* **61:** 545–550, 1997.