CKβ8, a novel CC chemokine that predominantly acts on monocytes

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Abstract We have studied the biological properties of a new human CC chemokine, CKβ8, consisting of 99 amino acids including six cysteines. CKβ8 mRNA transcripts were induced in monocytes by IL-1β and, to a lesser extent, by IFNγ, and were detected in RNA extracted from normal human liver and gastrointestinal tract. CKβ8 is chemotactic for monocytes, but is inactive on IL-2 conditioned T lymphocytes, eosinophils and neutrophils. Desensitization experiments indicate that CKβ8 and MIP-1β completely share receptors on monocytes and that the CKβ8 receptor, which appears to differ from the known ones, is also recognized by MCP-1, MCP-2, MCP-3, MCP-4, MIP-1α and RANTES.

Key words: Expression; Chemotaxis; Secretion; Receptor; Desensitization

1. Introduction

Chemokines are small proteins that regulate leukocyte recruitment in inflammation and immunity. They are characterized by four conserved cysteines linked to disulfide bonds and are subdivided into two groups, CXC and CC chemokines, depending on the arrangement of the first two cysteines which are separated by one amino acid or are adjacent [1–3]. CXC chemokines act mainly on neutrophils and activated T lymphocytes [3], while CC chemokines have a broader spectrum of activity and can attract monocytes [4,5], lymphocytes [6–8], basophils [9,10], and eosinophils [11,12]. We have studied the biological activities of a novel CC chemokine, CKβ8, that was identified within a large-scale sequencing program [13], and show here that CKβ8 acts predominantly on monocytes.

2. Materials and methods

2.1. Cloning and expression

The coding sequence of the novel chemokine was amplified by PCR from a human aortic endothelial cell cDNA library (Human Genome Sciences Inc., Rockville, MD). The cDNA was cloned into a baculovirus vector (BaculoGold™; Pharmingen, San Diego, CA), and the mature protein, CKβ8, was expressed in Sf9 insect cells (ATCC CRL 1711). The protein was purified from the supernatant of serum-free SF9 cell cultures in the presence of protease inhibitors (20 mg/ml Pefabloc SC; Boehringer, Mannheim, Germany, 1 mg/ml leupeptin, 1 mg/ml E64, and 1 mM EDTA) by three chromatography steps: cation exchange, heparin affinity, and size exclusion (poros 50 HS, poros 20 HE1, Perseptive Biosystem, and Sephacryl S200 HR; Pharmacia Fine Chemicals, Piscataway, NJ) [13].

2.2. Chemokines

MCP-1, MCP-2, MCP-3, RANTES, MIP-1α and MIP-1β were chemically synthesized by Dr. Ian Clark-Lewis (Biomedical Research Centre and Department of Biochemistry, University of British Columbia, Vancouver, Canada) according to established protocols [14]. MCP-4 was cloned and expressed as described [15].

2.3. Human cells and tissues

Monocytes [5], lymphocytes [16], and neutrophils [17] were isolated from donor-blood buffy coats. Lymphocytes were cultured in presence of IL-2 as previously described [16]. Fresh blood of healthy individuals was used to purify eosinophils by dextran sedimentation followed by Percoll density-gradient and negative selection with anti-CD16 monoclonal antibody (mAb)-coated magnetic beads [11]. For RNA extraction monocytes were used immediately after purification or following culture for 2, 8, 24, 48 h in the presence or absence of 10 ng/ml IL-1β or 100 U/ml IFNγ. Normal human tissues were collected for RNA extraction with permission of the Local Ethical Committee from brain-dead organ donors.

2.4. Northern blot analysis

Total RNA from human tissues and leukocytes was extracted by the acid guanidinium thiocyanate phenol-chloroform method [18] or the RNAzol B method as recommended by the supplier (Tel-Test Inc., Friendswood, TX). Samples of 10 μg RNA were fractionated on 1.2% denaturing agarose-formaldehyde gels, vacuum-transferred onto Nytran membranes and immobilized. RNA from LPS-stimulated monocytes was used to generate the probe for CKβ8 with the following primers: antisense 5′-TAA TCT AGA CTT CCT GGT CTT GAT CCG and sense 5′-TAT CTG CAG CCT CAT GCT TGT TAC TGT. The amplified PCR product was subcloned into pT7T3 18U plasmid (Pharmacia) and sequenced. This 346-bp PCR fragment, as well as the 206-bp fragment for IL-8 corresponding to the position 179 to 385 of the published cDNA 3-10C [19] and the 345-bp fragment for RANTES corresponding to the position 27 to 372 of the published cDNA [20] were labeled with [α-^{32}P]dATP using a random primer labeling kit (Boehringer Ltd., Mannheim, Germany). Hybridization was performed with 2×10^6 cpm/ml of the labelled probe in the presence of 50% formamide at 42°C for 18 h [21]. Membranes were washed to a stringency of 0.2×SSC, 0.1% SDS at 65°C and exposed to screens which were subsequently analyzed using a phosphorimager (Molecular Dynamics Inc., Sunnyvale, CA) and the ImageQuant software. After each hybridizatoin, the probe was removed by washing in 50% formamide, 0.1×SSC, 0.1% SDS at 65°C for 30 min.

2.5. In vitro chemotaxis

Chemotaxis was assessed in 48-well chambers (Neuro Probe, Cabin John, MD) using polyvinylpyrrolidone-free polycarbonate membranes (Nucleopore, Neuro Probe, Cabin John, MD) with 5-μm pores for monocytes, neutrophils and eosinophils, and 3-μm pores for lymphocytes as previously described [5,16,22]. All assays were done in triplicate, and the migrated cells were counted in five randomly selected fields at 1000-fold magnification. Spontaneous migration was determined in the absence of chemoattractant.

2.6. Enzyme release

The release of *N*-acetyl-β-D-glucosaminidase was tested in mono-

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Abbreviations: MIP, macrophage inflammatory protein; MCP, monocyte chemoattractant protein; RANTES, regulated on activation, normal T expressed and secreted; $[Ca^{2+}]_i$, cytosolic free calcium concentration; PTx, *Bordetella pertussis* toxin

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CKβ8      RVTKDAETEFMMSKLPLENPVLLDRFHATSADCCISYTPRSIPCSLLESYFETN.SEC
MIP-1α                                ASLAAD-PTACCF---S-Q--QNFIAD----S.-QC
MIP-1β                                APMGSDPPTACCF---A-KL-RNFVVD-Y--S.-LC
MCP-3                                 QPVGIN--TTCCYRFINKK--KQR----RR-TS-HC
I-309                                 SKSMQVPFSRCCF-FAEQE--LRAILC-RN-S.-IC

CKβ8      SKPGVIFLTKKGRRFCANPSDKQVQVCMRMLKLDTRIKTRKN
MIP-1α    ----------RS-QVC-D--EEW--KYVSD-E-SA
MIP-1β    -Q-A-V-Q--RSKQVC-D--ESW--EYVYD-E-N
MCP-3     PREA---K--LDKEIC-D-TQ-W--DF-KH-DKK-QTPKL
I-309     -NE-L--KL-R-KEAC-LDTVGW--RHRK--RHCPSKRK
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Fig. 1. Amino acid sequence of CKβ8 aligned with MIP-1α, MIP-1β, MCP-3 and I-309. Identical amino acids are represented by hyphens.

cytes. In brief, samples of 1.2×10^6 monocytes in 0.3 ml of prewarmed medium (136 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1 mM $CaCl_2$, 20 mM Hepes, pH 7.4, 5 mM D-glucose, and 1 mg/ml fatty acid-free BSA) were pretreated for 2 min with cytochalasin B (2.7 mg/ml) and then stimulated with a chemokine. The reaction was stopped after 3 min by cooling on ice and centrifugation, and the enzyme activity was determined in the supernatant [5]. The release of elastase was tested in neutrophils as described previously [17].

2.7. Cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) changes

Monocytes, eosinophils, neutrophils and lymphocytes loaded with Fura-2 (0.2 nmol/10^6 cells) were stimulated with a chemokine, and $[Ca^{2+}]_i$-related fluorescence changes were recorded [23]. The same asssay was used to test receptor desensitization in monocytes after repeated chemokine stimulation at 90-s intervals [5].

2.8. Treatment with B. pertussis toxin (PTx)

Monocytes (10^7 cells/ml in 130 nM NaCl, 4.6 mM KCl, 5.0 mM $NaHCO_3$, 0.05 mM $CaCl_2$, 20 mM Hepes (pH 7.4), and 5 mM D-glucose) were incubated for 90 min in the presence or absence of 2 mg/ml PTx. Cells were then washed, resuspended in 136 mM NaCl, 4.8 mM KCl, 1 mM $CaCl_2$, 5 mM glucose, 20 mM Hepes (pH 7.4), and immediately used for analysis of $[Ca^{2+}]_i$ changes [24].

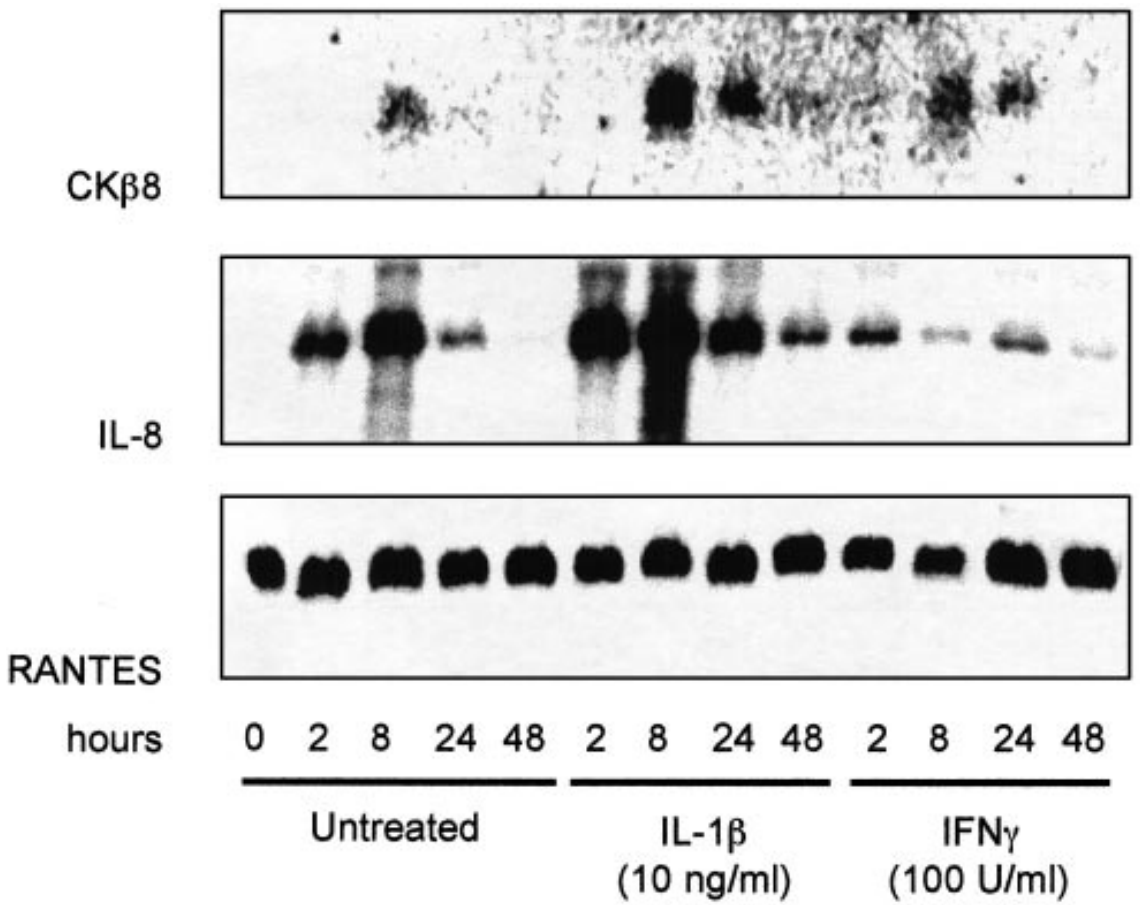

Fig. 2. CKβ8, IL-8 and RANTES expression on monocytes. Total RNA from human monocytes was analyzed at different time points. Cells were cultured in the presence or absence of IL-1β or IFNγ. A representative experiment out of five is shown.

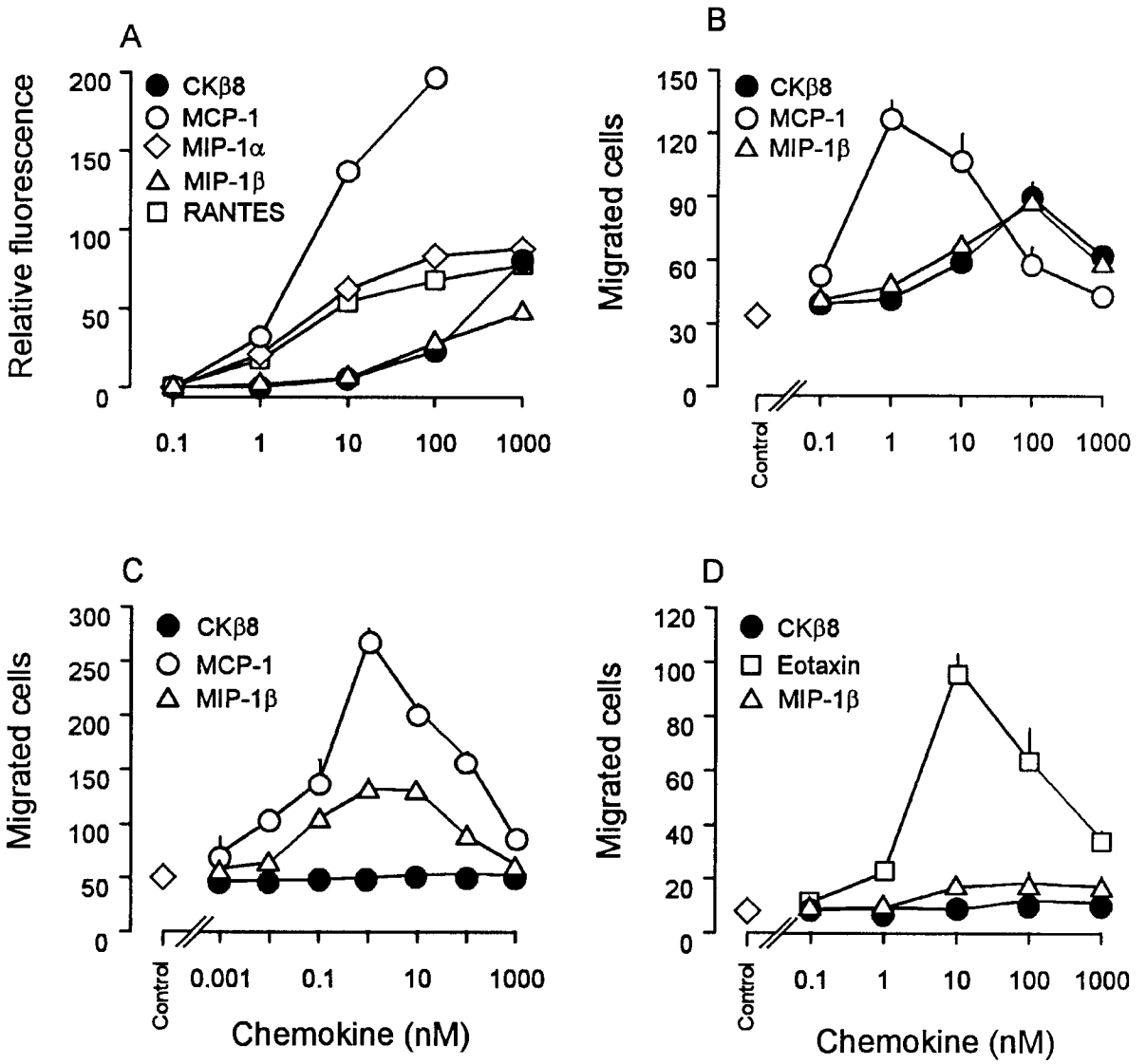

Fig. 3. A: Release of *N*-acetyl-β-D-glucosaminidase from cytochalasin B-treated human blood monocytes in response to CKβ8 and other CC chemokines. Enzyme activity is presented in arbitrary fluorescence units. One out of three similar experiments performed with cells from different donors is shown. B-D: Chemotactic responses of human monocytes (B), IL-2 conditioned T lymphocytes (C) and eosinophils (D). Numbers of migrating cells per five high-power fields are given. Mean ± SEM of five experiments performed with cells from different donors.

3. Results

3.1. CKβ8 sequence

The purified chemokine (see Section 2) yielded a single band with an apparent molecular mass of 11 kDa and no evidence for contaminant proteins on SDS-PAGE. Laser desorption mass spectrometry resolved a single peak with a molecular mass of 11 284 ± 30. The protein consists of 99 amino acids and has an unusually long NH_2-terminal domain of 32 amino acids preceding the first cysteine. Amino acid identities between CKβ8 and the reference chemokines are 50% for MIP-1α, 41% for MIP-1β, 37% for MCP-3, 33% for MCP-1, 32% for I-309, and 31% for RANTES. CKβ8 contains two cysteines in addition to the four conserved ones (Fig. 1).

3.2. Expression

As shown in Fig. 2, CKβ8 mRNA expression was transiently induced in monocytes by adherence with a maximum at 8 h. Stimulation with either IL-1β or IFNγ increased CKβ8 mRNA levels with a similar time course. The effect of IL-1β, however, was considerably more pronounced than that of IFNγ. The expression of IL-8 and RANTES was also assessed. Transcripts for IL-8 were induced with a similar time course by IL-1β, but not by IFNγ which was inhibitory. By contrast, RANTES was constitutively expressed and no changes were observed upon stimulation. Expression of CKβ8 was also tested by Northern analysis of RNA extracted from lung, pleura, thymus, esophagus, stomach, duodenum, jejunum, ileum, colon, pancreas, liver, spleen, kidney and urinary bladder. Positive signals were obtained for liver, jejunum, ileum, and colon (data not shown).

3.3. Enzyme release in monocytes

The activity of CKβ8 was tested on monocytes, by measuring the release of the lysosomal enzyme *N*-acetyl-β-D-glucosaminidase, a test that is particularly suited for quantitative

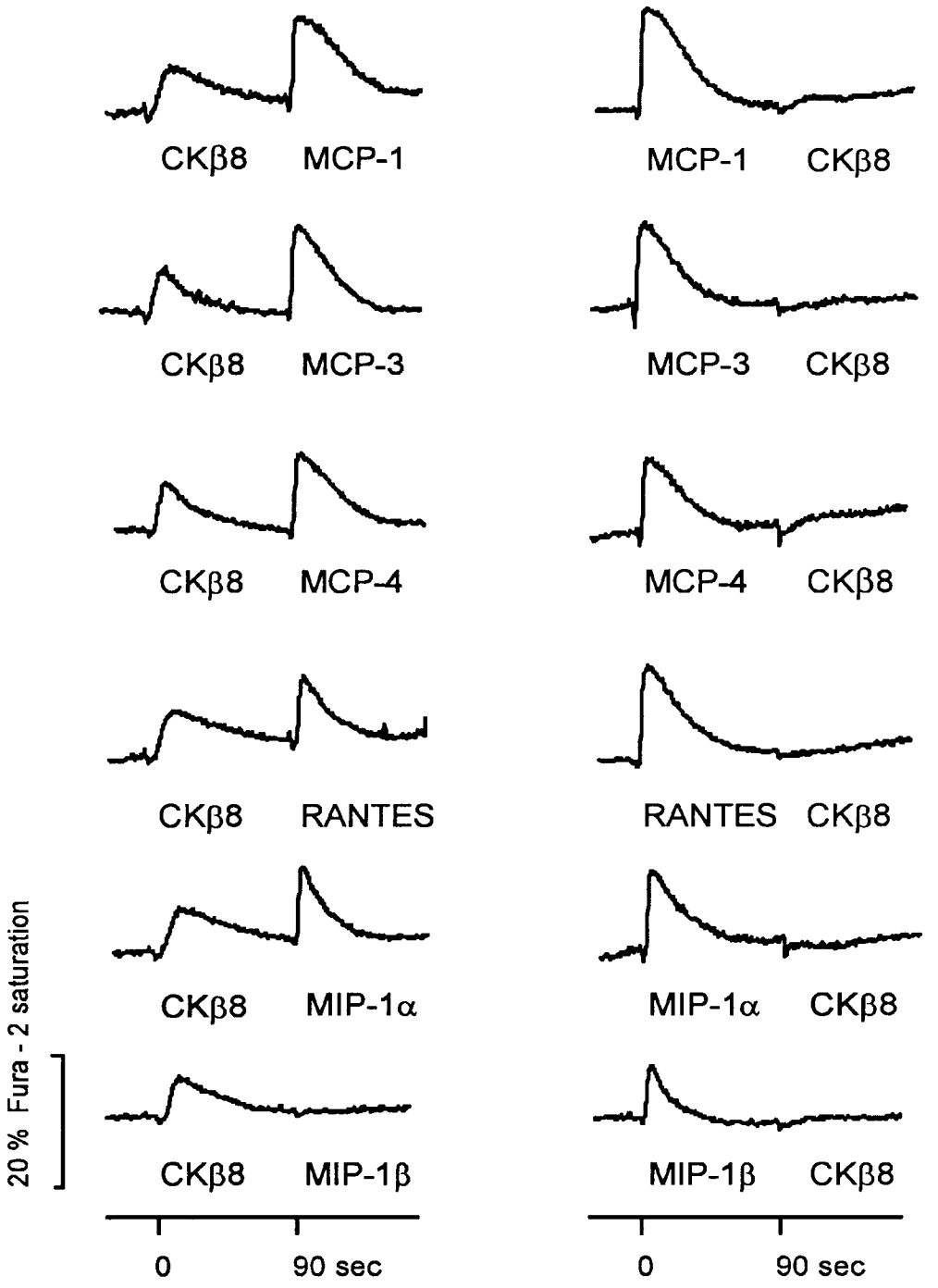

Fig. 4. Cross-desensitization of human blood monocytes. Fura-2-loaded monocytes were stimulated sequentially at 90-s intervals with 100 nM CKβ8 and other CC chemokines, and $[Ca^{2+}]_i$-dependent fluorescence changes were recorded. The tracings are representative for three separate experiments that were performed under identical conditions with cells from different donors.

purposes [5]. As shown in Fig. 3A, the effect of CKβ8 was concentration dependent with a threshold at 10 nM and a steady increase up to 1000 nM. The activity was comparable to that of MIP-1β, but was clearly lower than that of MIP-1α and RANTES, and MCP-1 which is a very potent stimulus of exocytosis. Like MIP-1β and all the other CC chemokines, CKβ8 did not induce elastase release in neutrophils (data from three independent experiments, not shown).

3.4. In vitro chemotaxis

The chemotactic activity of CKβ8 was tested in monocytes, lymphocytes and eosinophils (Fig. 3B–D). A significant migration response was obtained only with monocytes where the activity of CKβ8 was identical in terms of efficacy and potency to that of MIP-1β. In agreement with former studies [5], MCP-1 was more potent. CKβ8 was completely inactive on IL-2 conditioned lymphocytes, which responded to MCP-1 and MIP-1β, eosinophils which showed the expected response to eotaxin, and neutrophils (data not shown).

3.5. Calcium mobilization

A rapid and transient rise in $[Ca^{2+}]_i$ was observed after

CKβ8 stimulation of monocytes. The rate and the magnitude of the rise increased with the concentration. The threshold of calcium mobilization was approximately 30 nM and maximum values were obtained at chemokine concentrations of 100 nM. In agreement with the lack of functional activities CKβ8 did not induce $[Ca^{2+}]_i$ changes in lymphocytes, eosinophils, or neutrophils up to a concentration of 100 nM.

3.6. Receptor desensitization

Receptor usage by CKβ8 and other CC chemokines was assessed in monocytes by monitoring $[Ca^{2+}]_i$ changes after sequential stimulation. As shown in Fig. 4, treatment of monocytes with MCP-1, MCP-2 (not shown), MCP-3, MCP-4, RANTES, MIP-1α and MIP-1β abolished responsiveness to CKβ8. Prestimulation with CKβ8 did not affect the response of the MCPs, and only slightly reduced the response of RANTES and MIP-1α. In contrast CKβ8 completely desensitized the cells toward MIP-1β. These results indicate that CKβ8 and MIP-1β completely share receptors on monocytes, and that all the other chemokines tested recognize the receptor that mediates the activity of CKβ8 on monocytes.

We have cultured monocytes for 24 h under standard conditions in RPMI supplement with 10% FCS, and have found that desensitization of the response of MIP-1β by CKβ8 was largely lost (data not shown). This experiment suggests the up-regulation of a receptor that recognizes MIP-1β but not CKβ8, possibly CCR5.

3.7. Effect of B. pertussis toxin

Pretreatment of monocytes with 2 μg/ml PTx for 90 min at 37°C completely abrogated $[Ca^{2+}]_i$ changes induced by 100 nM CKβ8 or MIP-1β, suggesting that the receptor shared by these chemokines is coupled to PTx-sensitive G proteins. The PTx pretreatment also virtually abrogated the $[Ca^{2+}]_i$ changes elicited by MCP-1 which was used as control (data not shown) in agreement with former observations [25].

4. Discussion

We have described a CC chemokine, CKβ8, that is structurally and functionally related to MIP-1α and MIP-1β and acts on human monocytes, but not on eosinophils, neutrophils and IL-2 conditioned T lymphocytes. The same chemokine was recently shown to inhibit colony formation by myeloid progenitor cells, and was named myeloid progenitor inhibitory factor 1 (MPIF-1) [13].

CKβ8 has an unusually long NH_2-terminal domain of 32 amino acids preceding the first two conserved cysteines. Like the CC chemokine I-309, CKβ8 has two cysteines in addition to the four conserved ones, but they are located at different positions. I-309 was also reported to act specifically on monocytes [26]. Three CC chemokines with long NH_2-terminal domain and six cysteines at corresponding positions, C10/MRP-1 [27], MIP-1τ [28] and CCF-18/MRP-2 [29,30], were previously described in mice. They show a moderate degree of sequence identity with CKβ8 (33–37%). These murine chemokines were shown to inhibit colony formation by myeloid progenitors cells, an activity that they share with human MIP-1α and CKβ8.

The expression of CKβ8, as assessed by Northern blotting in PBL differs, at least in part, from that of IL-8 and RANTES. CKβ8 mRNA was induced upon adherence to plastic and was enhanced by IFNγ and much more efficiently by IL-1β. Expression of IL-8 was also enhanced by IL-1β in agreement with previous reports [31,32], but not by IFNγ as previously shown in monocytes [33,34] and granulocytes [35]. Under similar conditions RANTES was expressed constitutively and the level of transcripts was not affected by IL-1β nor IFNγ [36].

The similarity of the response of monocytes to CKβ8 and MIP-1β and the results of the desensitization experiments suggest that CKβ8 acts via a novel receptor. Since the $[Ca^{2+}]_i$ changes induced by CKβ8 were abrogated by pretreatment with MCP-1, MCP-3, MCP-4, RANTES, MIP-1α and MIP-1β. the CKβ8 receptor appears to recognize most of the CC chemokines. A complete cross-desensitization was observed on monocytes, only between CKβ8 and MIP-1β. This observation could be taken to suggest that CCR5, the only receptor positively known to bind MIP-1β, might also bind CKβ8. This hypothesis, however, must be rejected because CKβ8 was inactive on IL-2 conditioned T lymphocytes [16], and did not prevent the $[Ca^{2+}]_i$ changes induced by MIP-1β in monocytes cultured for 24 h to up-regulate CCR5 [37]. Since CKβ8 did not induce $[Ca^{2+}]_i$ changes or chemotaxis in lymphocytes and eosinophils, there is strong evidence that it does not act via CCR1, CCR2, CCR3 or CCR5. Further evidence for this conclusion stems from the data on monocytes, where CKβ8 pretreatment had little effect on the subsequent stimulation with MCPs, RANTES or MIP-1α. CCR4 can be also excluded as receptor for CKβ8 based on the report of Power et al. [38], showing that MIP-1β does not induce $[Ca^{2+}]_i$ mobilization on CCR4 transfectants.

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References

[1] J.J. Oppenheim, C.O.C. Zachariae, N. Mukaida, K. Matsushima, Annu. Rev. Immunol. 9 (1991) 617–648.
[2] M. Baggiolini, B. Dewald, B. Moser, Adv. Immunol. 55 (1994) 97–179.
[3] T.J. Schall, K.B. Bacon, Curr. Opin. Immunol. 6 (1994) 865–873.
[4] J. Van Damme, P. Proost, J.-P. Lenaerts, G. Opdenakker, J. Exp. Med. 176 (1992) 59–65.
[5] M. Uguccioni, M. D'Apuzzo, M. Loetscher, B. Dewald, M. Baggiolini, Eur. J. Immunol. 25 (1995) 64–68.
[6] T.J. Schall, K. Bacon, K.J. Toy, D.V. Goeddel, Nature 347 (1990) 669–671.
[7] D.D. Taub, K. Conlon, A.R. Lloyd, J.J. Oppenheim, D.J. Kelvin, Science 260 (1993) 355–358.
[8] P. Loetscher, M. Seitz, I. Clark-Lewis, M. Baggiolini, B. Moser, FASEB J. 8 (1994) 1055–1060.
[9] R. Alam, P.A. Forsythe, S. Stafford, M.A. Lett-Brown, J.A. Grant, J. Exp. Med. 176 (1992) 781–786.
[10] Y. Tanimoto, K. Takahashi, I. Kimura, Clin. Exp. Allergy 22 (1992) 1020–1025.
[11] A. Rot, M. Krieger, T. Brunner, S.C. Bischoff, T.J. Schall, C.A. Dahinden, J. Exp. Med. 176 (1992) 1489–1495.
[12] M. Baggiolini, C.A. Dahinden, Immunol. Today 15 (1994) 127–133.
[13] V.P. Patel, B.L. Kreider, Y. Li, H. Li, K. Leung, T. Salcedo, B.

Nardelli, V. Pippalla, S. Gentz, R. Thotakura, D. Parmelee, R. Gentz, G. Garotta, J. Exp. Med. 185 (1997) 1163–1172.
[14] I. Clark-Lewis, B. Moser, A. Walz, M. Baggiolini, G.J. Scott, R. Aebersold, Biochemistry 30 (1991) 3128–3135.
[15] M. Uguccioni, P. Loetscher, U. Forssmann, B. Dewald, H.D. Li, S.H. Lima, Y.L. Li, B. Kreider, G. Garotta, M. Thelen, M. Baggiolini, J. Exp. Med. 183 (1996) 2379–2384.
[16] P. Loetscher, M. Seitz, M. Baggiolini, B. Moser, J. Exp. Med. 184 (1996) 569–577.
[17] P. Peveri, A. Walz, B. Dewald, M. Baggiolini, J. Exp. Med. 167 (1988) 1547–1559.
[18] P. Chomczynski, N. Sacchi, Anal. Biochem. 162 (1987) 156–159.
[19] J. Schmid, C. Weissmann, J. Immunol. 139 (1987) 250–256.
[20] T.J. Schall, J. Jongstra, B.J. Dyer, J. Jorgensen, C. Clayberger, M.M. Davis, A.M. Krensky, J. Immunol. 141 (1988) 1018–1025.
[21] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular cloning: a laboratory manual. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press, 1989.
[22] M. Weber, M. Uguccioni, B. Ochensberger, M. Baggiolini, I. Clark-Lewis, C.A. Dahinden, J. Immunol. 154 (1995) 4166–4172.
[23] V. Von Tscharner, B. Prod'hom, M. Baggiolini, H. Reuter, Nature 324 (1986) 369–372.
[24] S.A. Jones, B. Moser, M. Thelen, FEBS Lett. 364 (1995) 211–214.
[25] S. Sozzani, D. Zhou, M. Locati, M. Rieppi, P. Proost, M. Magazin, N. Vita, J. Van Damme, A. Mantovani, J. Immunol. 152 (1994) 3615–3622.
[26] M.D. Miller, M.S. Krangel, Proc. Natl. Acad. Sci. USA 89 (1992) 2950–2954.
[27] A. Orlofsky, M.S. Berger, M.B. Prystowsky, Cell Regul. 2 (1991) 403–412.
[28] A.N. Poltorak, F. Bazzoni, I.I. Smirnova, E. Alejos, P. Thompson, G. Luheshi, N. Rothwell, B. Beutler, J. Inflamm. 45 (1995) 207–219.
[29] T. Hara, K.B. Bacon, L.C. Cho, A. Yoshimura, Y. Morikawa, N.G. Copeland, D.J. Gilbert, N.A. Jenkins, T.J. Schall, A. Miyajima, J. Immunol. 155 (1995) 5352–5358.
[30] B.-S. Youn, I.-K. Jang, H.E. Broxmeyer, S. Cooper, N.A. Jenkins, D.J. Gilbert, N.G. Copeland, T.A. Elick, M.J. Fraser Jr., B.S. Kwon, J. Immunol. 155 (1995) 2661–2667.
[31] K. Kasahara, R.M. Strieter, T.J. Standiford, S.L. Kunkel, Pathobiology 61 (1993) 57–66.
[32] P. Rathanaswami, M. Hachicha, M. Sadick, T.J. Schall, S.R. McColl, J. Biol. Chem. 268 (1993) 5834–5839.
[33] S. Schnyder-Candrian, R.M. Strieter, S.L. Kunkel, A. Walz, J. Leukocyte Biol. 57 (1995) 929–935.
[34] G.L. Gusella, T. Musso, M.C. Bosco, I. Espinoza-Delgado, K. Matsushima, L. Varesio, J. Immunol. 151 (1993) 2725–2732.
[35] M.A. Cassatella, S. Gasperini, F. Calzetti, P.P. McDonald, G. Trinchieri, Biochem. J. 310 (1995) 751–755.
[36] T.J. Schall, Cytokine 3 (1991) 165–183.
[37] C. Combadiere, S.K. Ahuja, H.L. Tiffany, P.M. Murphy, J. Leukocyte Biol. 60 (1996) 147–152.
[38] C.A. Power, A. Meyer, K. Nemeth, K.B. Bacon, A.J. Hoogewerf, A.E.I. Proudfoot, T.N.C. Wells, J. Biol. Chem. 270 (1995) 19495–19500.