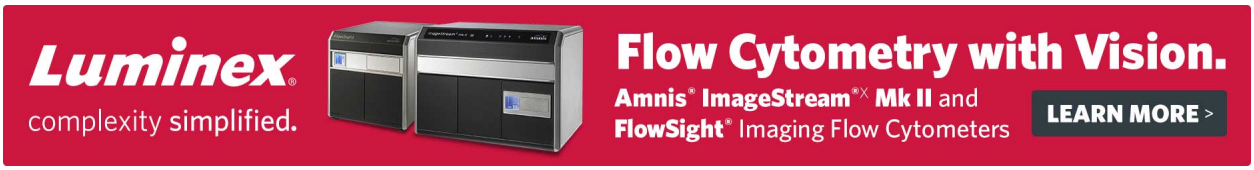

The Journal of Immunology

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J Immunol 1998; 160:2418-2424; ;
http://www.jimmunol.org/content/160/5/2418

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Print ISSN: 0022-1767 Online ISSN: 1550-6606.

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CKβ-11/Macrophage Inflammatory Protein-3β/EBI1-Ligand Chemokine Is an Efficacious Chemoattractant for T and B Cells[1]

Chang H. Kim,*† Louis M. Pelus,‡ John R. White,§ Edward Applebaum,‖ Kyung Johanson,# and Hal E. Broxmeyer[2]*†

We examined the functional properties of CKβ-11/MIP-3β/ELC, a recently reported CC chemokine that specifically binds to a chemokine receptor, EBI1/BLR2/CCR7. CKβ-11/MIP-3β/ELC is distantly related to other CC and CXC chemokines in primary amino acid sequence structure. Recombinant human CKβ-11/MIP-3β/ELC expressed from a mammalian cell system showed potent chemotactic activity for T cells and B cells but not for granulocytes and monocytes. An optimal concentration of CKβ-11/MIP-3β/ELC attracted most input T cells within 3 h, a chemotactic activity comparable with that of stromal cell derived factor 1 (SDF-1), a highly efficacious CXC chemokine. CKβ-11/MIP-3β/ELC equally attracted naive CD45RA$^+$ and memory type CD45RO$^+$ T cells. CKβ-11/MIP-3β/ELC also strongly attracted both CD4$^+$ and CD8$^+$ T cells, but the attraction for CD4$^+$ T cells was greater. CKβ-11/MIP-3β/ELC was also a more efficacious chemoattractant for B cells than MIP-1α, a known B cell chemoattractant. CKβ-11/MIP-3β/ELC induced actin polymerization in lymphocytes, and chemotaxis was completely blocked by pertussis toxin showing its receptor, most likely EBI1/BLR2/CCR7, is coupled to a $G_{\alpha i}$ protein. CKβ-11/MIP-3β/ELC induced calcium mobilization in lymphocytes, which could be desensitized by SDF-1, suggesting possible cross-regulation in their signaling. Human CKβ-11/MIP-3β/ELC attracted murine splenocytes suggesting functional conservation of CKβ-11/MIP-3β/ELC between human and mouse. The efficacy of chemoattraction by CKβ-11/MIP-3β/ELC and tissue expression of its mRNA suggest that CKβ-11/MIP-3β/ELC may be important in trafficking of T cells in thymus, and T cell and B cell migration to secondary lymphoid organs. ***The Journal of Immunology,*** **1998, 160: 2418–2424.**

Members of the chemokine superfamily are small peptide molecules with molecular mass of ~10 kDa, activating, suppressing, and attracting cells with relatively specific activities. Most chemokines belong to either the CC or CXC family, depending on the spacing between the first two cysteine residues (1–4). Two recently cloned chemoattractants, lymphotactin and fractalkine, do not fit into this conventional grouping. Lymphotactin is missing the first and third cysteine residues (5), and fractalkine is membrane bound and has a CX_3C motif (6, 7). In humans, the CC chemokine genes are found on chromosome 17, while genes of CXC chemokines are clustered on chromosome 4. However, genes for the human fractalkine (6) and thymus- and activation-regulated chemokine (8) are found on chromosome 16, and genes for the human lymphotactin (5) and liver- and activation-regulated chemokine (9) are found on chromosomes 1 and 2, respectively. The primary function of chemokines appears to be the chemoattraction of various cells, especially leukocytes, in a haptotactic gradient-dependent fashion. Depending on the type of target cells and place of action, chemokines can be involved in diverse biologic processes such as inflammation, angiogenesis, regulation of cell proliferation and maturation, and leukocyte homing or migration. Some chemokines, such as IL-8, MIP-1β[3] and RANTES, are reported to modulate integrin adhesion and thought to be important in migration of cells from one environment to another (10–13). SDF-1, a CXC chemokine, has been reported to attract lymphocytes, monocytes, and hemopoietic progenitor cells (14–16, 47) and, thus far, appears to be one of the most efficacious chemoattractants for T cells among known CC and CXC chemokines.

CKβ-11 was identified as an expressed sequence tag (EST) from a human fetal spleen library by Human Genome Sciences (Rockville, MD). This chemokine was expressed in mammalian cells and the resulting protein was characterized using various leukocyte cell populations. MIP-3β (17) and EBI1-ligand chemokine (ELC) (18) were recently reported to be identical to CKβ-11 and the ligand for BLR2/EBI1/CCR7 (18). The gene for human CKβ-11/MIP-3β/ELC was found on chromosome 9 and its mRNA was detected in thymus, lymph nodes, lung, and intestine (18). We report here that CKβ-11/MIP-3β/ELC is a strong chemoattractant for T cells expressing CD4, CD8, CD45RO, and CD45RA, and for mature B cells, but not for monocytes and granulocytes. It also stimulates

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Received for publication July 22, 1997. Accepted for publication November 10, 1997.

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[1] This work was supported by Public Health Service Grants R01 HL 56416 and R01 HL 54037 and by a project in P01 HL 53586 from the National Institutes of Health (Bethesda, MD) to H.E.B.

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[3] Abbreviations used in this paper: MIP, macrophage inflammatory protein; PE, phycoerythrin; MCP-1, monocyte chemoattractant protein-1; CCR, CC chemokine receptor; SDF-1, stromal cell-derived factor 1; ELC, EBI1-ligand chemokine; BLR, Burkitt's lymphoma receptor; sIgM, surface immunoglobulin M; CHO, Chinese hamster ovary; MALDI, matrix-assisted laser desorption ionized; ELR, Glu-Leu-Arg.

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actin polymerization in lymphocytes and its signaling for chemotaxis is abolished by pertussis toxin. Calcium mobilization by CKβ-11/MIP-3β/ELC in lymphocytes is desensitized by another efficacious chemoattractant, SDF-1, suggesting a possibility of cross-regulation of signaling between the two efficacious lymphocyte chemoattractants.

Materials and Methods

Expression of CKβ-11/MIP-3β/ELC

CKβ-11/MIP-3β/ELC was initially identified at Human Genome Sciences by random sequencing of expressed sequence tags in a cDNA library from human fetal spleen. A full length clone was subsequently identified and sequenced. CKβ-11/MIP-3β/ELC was expressed in Chinese hamster ovary (CHO) cells. For subcloning into an expression vector, the CKβ-11/MIP-3β/ELC coding region was amplified by PCR using the cDNA clone as substrate and the following pair of 5′-TC CCC GCG GCC ACC ATG GCC CTG CTA CTG GCC-3′ (*Sac*II site underlined and initiator methionine codon in bold) and 5′-GC TCT AGA CTA TCA GCG CCC TGG GCC ACG CTG GAT ACG GAT ACT TTT GCT CAA TGC TTG ACT CGG ACT-3′ (*Xba*I site underlined) oligonucleotide primers. The PCR product was digested with *Sca*II and *Xba*I, subcloned into an intermediate vector, and sequenced, and then recloned into mammalian cell expression vector pCDN (19). The resulting plasmid was linearized with *Pvu*I and introduced into a CHO cell line derivative by electroporation, and a polyclonal population of transformed cells with amplified vector copy number was selected by growth medium lacking nucleotides and containing methotrexate (20). Medium from the CHO stable cell line was used for purification of CKβ-11/MIP-3β/ELC.

Purification and analytical characterization of CKβ-11/MIP-3β/ELC from CHO-conditioned media

Purification, N-terminal analysis, and matrix-assisted laser desorption ionized (MALDI) mass spectrometry for CKβ-11/MIP-3β/ELC was conducted as described by Berkhout et al. (21) except that CKβ-11/MIP-3β/ELC-conditioned medium was substituted for MCP-4-conditioned medium. N-terminal sequence analysis of the CHO-derived purified CKβ-11/MIP-3β/ELC showed the N-terminal sequence of the mature secreted protein to begin at glycine-22. MALDI-mass spectrometry showed the major peak (8182 Da) to be lower in mass than the expected 8800 Daltons, suggesting that proteolytic cleavage had resulted in loss of five amino acids from the C terminus of the expected 77-amino acid protein.

Abs and chemokines

mAbs, conjugated with fluorescent dyes, FITC, phycoerythrin (PE), or tricolor, to human CD3 (clone S4.1), CD8 (clone 3B5), CD45RA (clone MEM56), CD45RO (clone UCHL1), and CD19 (clone SJ25-C1) were obtained from Caltag (Burlingame, CA). PE-conjugated mAb to human CD4 (clone SK3) was obtained from Becton Dickinson (San Jose, CA). SDF-1 was a kind gift from Dr. Ian Clark-Lewis (University of British Columbia, Vancouver, Canada). MIP-1α, MIP-1β, RANTES, monocyte chemoattractant protein (MCP)-1, and IL-8 were purchased from R&D Systems (Minneapolis, MN).

Cell isolation

Peripheral blood buffycoat was obtained from the Central Indiana Regional Blood Center (Indianapolis, IN), diluted 1:3, layered on Ficoll-Paque (1.077 g/ml) (Biochem KG, Berlin, Germany), and centrifuged for separation of low density mononuclear cells from RBC and polymorphonuclear cells. Bone marrow aspirates were obtained from healthy donors after receiving informed consent. Aspirates were diluted 1:2 with PBS (pH 7.4) and layered on Ficoll-Paque for centrifugation. Mononuclear cells were collected from the interphase of Ficoll-Paque and serum layers and washed twice with PBS (pH 7.4).

For lymphocytes, mononuclear cells were incubated overnight in plastic culture flasks to remove adherent monocytes. For total leukocytes containing granulocytes, 1 ml of peripheral blood buffycoat was added to 9 ml of hypotonic NH_4Cl buffer and incubated at room temperature for 5 min to lyse RBCs. The cells were washed twice with PBS (pH 7.4).

In vitro two-chamber migration assay for leukocytes

Chemokine-dependent chemotaxis was assayed on various leukocytes by an in vitro two-chamber migration assay followed by flow cytometry (15, 22). One hundred microliters of cells in RPMI 1640 medium supplemented with 0.5% BSA was added to the upper chamber of Costar Transwells (6.5 mm diameter, 5-μm pore size, polycarbonate membrane), and chemokines were added to the upper and/or lower chamber to form various chemokine gradients. A total of 5×10^5 mononuclear cells were added to the upper chamber of the Transwell and incubated 2 h for monocyte migration and 3 h for lymphocyte migration. After collecting cells in suspension, 0.5 ml of 5 mM EDTA was added to the lower chamber for 15 min at 37°C to detach adherent cells such as monocytes and granulocytes from the bottom of wells. Detached cells were combined with the previously collected suspension cells for cell counting. Migrated monocytes and lymphocytes were counted by FACscan (Becton Dickinson) for 20 s by gating on appropriate populations of cells using forward-scatter and side-scatter channels. For counting $CD3^+$ T cells and $CD19^+$ B cells, migrated lymphocytes were stained with mAbs to CD3 and CD19, respectively conjugated with FITC and PE (Caltag), and $CD3^+CD19^-$ T cells and $CD3^-CD19^+$ B cells were counted by FACscan for 20 s. The amount of all mAbs used to stain migrated cells in each well was 500 ng in 50 μl staining buffer (1% BSA and 0.01% NaN_3 in PBS, pH 7.4). For $CD45RA^+$ and $CD45RO^+$ T cell subtypes, migrated lymphocytes in the lower chamber were three-color stained with fluorescent mAbs to CD45RA, CD45RO, and CD3. Numbers of $CD3^+CD45RA^+$ or $CD3^+CD45RO^+$ cells were counted for 20 s, or each cell population was collected to 10,000 events by FACscan for immunophenotyping of migrated and input cells. For counting $CD4^+$ or $CD8^+$ T cell subtypes by FACscan, cells migrated to the lower chamber were stained with mAbs to CD4 and CD8 Ags. For granulocytes, 5×10^5 peripheral blood cells after RBC lysis were added to the upper chamber of Costar Transwell (6.5 mm diameter, 3 μm pore size, polycarbonate membrane) and allowed 90 min for migration. Migrated granulocytes, obtained by collecting suspended cells and detaching adherent cells from the bottom of wells, were counted for 20 s by forward and side-scatter gating to exclude lymphocytes and monocytes. Each chemotaxis experiment was performed in duplicate. All data were analyzed by Student's *t* test for significance ($p < 0.05$), and representative results of at least three independent experiments were obtained.

Calcium flux responses in lymphocytes

Lymphocytes depleted of granulocytes and monocytes were freshly purified from peripheral blood buffycoat for each experiment (see above for details). Cells washed with PBS were loaded with 2.5 μM FURA-2 AM in HBSS (Sigma Chemical Co., St. Louis, MO, pH 7.4) supplemented with 0.05% BSA at 37°C for 45 min, and washed twice with PBS. FURA-2 AM-loaded cells were resuspended in HBSS supplemented with 0.05% BSA at 5×10^6 cells/ml, and placed in a continuously stirred cuvette at 37°C in a MSIII fluorometer (Photon Technology, South Brunswick, NJ). Fluorescence was monitored at 340 and 380 nm for excitation and 510 nm for emission. The data were recorded as the relative ratio of fluorescence excited at 340 and 380 nm. Data were collected every second.

Actin polymerization assay

T cells were resuspended in RPMI 1640 supplemented with 0.1% BSA at 1.25×10^6 cells/ml. CKβ-11/MIP-3β/ELC was added at the indicated concentration to the cell solution, and at 15 s post-treatment with CKβ-11/MIP-3β/ELC (the 15-s time point was found to be the peak time point for actin polymerization by CKβ-11/MIP-3β/ELC in preliminary experiments), 0.4 ml of cell solution was transferred to 0.1 ml of FITC-labeled phalloidin solution (4×10^{-7} M FITC-labeled phalloidin, 0.5 mg/ml 1-α-lysophosphatidylcholine, and 18% formaldehyde in PBS, all from Sigma Chemical Co.) to stain and fix cells. Cells were incubated for 10 min, pelleted, and resuspended in 0.5 ml of 1% paraformaldehyde solution. Mean fluorescence was measured by FACscan.

Results

Chemotactic activity of CKβ-11/MIP-3β/ELC for T cells

CKβ-11/MIP-3β/ELC showed potent chemotactic activity toward peripheral blood lymphocytes (Table I). Maximal attraction at the concentrations assessed was observed at CKβ-11/MIP-3β/ELC concentrations between 200 and 2000 ng/ml. Chemokinetic activity, defined as a random movement induced by chemoattractants in a zero gradient (containing equal amounts of starting chemoattractant in both chambers), was low (Table I). We stained the migrated lymphocytes with anti-CD3 Ab to specifically count T cells and rule out the effect of CKβ-11/MIP-3β/ELC on non-T cells. MIP-1α and MCP-1 were often too weak to attract T cells significantly, while CKβ-11/MIP-3β/ELC attracted approximately 90%

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Table I. *Checkerboard assay of CKβ-11/MIP-3β/ELC on lymphocytes*[a]

CKβ-11 Concentration (ng/ml) in Lower Chamber	CKβ-11 Concentration (ng/ml) in Upper Chamber			
	0	20	200	2000
0	8.1 (2.4)	6.9 (3.2)	5.9 (1.2)	6.6 (1.6)
20	9.3 (2.3)	7.3 (0.6)	7.4 (1.6)	5.8 (1.0)
200	66.1 (7.0)	64.8 (6.4)	6.6 (0)	6.5 (1.4)
2000	69.6 (4.6)	76.4 (5.6)	54.0 (1.4)	21.9 (0.4)

[a] Results are expressed as percentage of input lymphocytes (range of duplicates) and representative of three experiments.

of input T cells (Fig. 1). CKβ-11/MIP-3β/ELC attracted both $CD4^+CD8^-$ and $CD4^-CD8^+$ T cells better than other chemokines such as MCP-1 and MIP-1α (Fig. 2). Although, CKβ-11/MIP-3β/ELC is an efficacious chemoattractant for both $CD4^+$ helper and $CD8^+$ cytotoxic T cells, the chemotactic activity for $CD4^+$ cells (78% maximum net migration over background) was slightly greater than for $CD8^+$ cytotoxic T cells (58% maximum net migration) (Fig. 2, *A* and *B*). CKβ-11/MIP-3β/ELC demonstrated strong chemotactic activity for $CD45RA^+$ and $CD45RO^+$ T cells with no significant preference for either subtype, while consistent with reports of others (23, 24), MCP-1 and MIP-1α showed preference for $CD45RO^+$ cells with optimum concentrations, 10 to 1000 ng/ml for MCP-1 and 100 to 1000 ng/ml for MIP-1α (Fig. 3, *A*, *B*, and *C*).

Chemotactic activity of human CKβ-11/MIP-3β/ELC on human B cells and mouse splenocytes, but not on human monocytes and granulocytes.

IL-8, Gro-α, and MIP-1α have been reported to attract B cells (25, 26). To specifically monitor $CD19^+$ B cell migration in response to chemokines, we stained input and migrated cells with fluorescent Abs to CD3 and CD19 and counted $CD3^-CD19^+$ cells. CKβ-11/MIP-3β/ELC usually attracted 15 to 30% of input $CD3^-CD19^+$ B cells at chemokine concentrations between 10 and 100 ng/ml (Fig. 4*A*). This was a stronger attraction than that for MIP-1α. We examined the effect of CKβ-11/MIP-3β/ELC on immature $CD34^+CD19^+$ pro/pre-B cell progenitors in bone marrow and found that it had no activity on these cells (data not shown). We used also mAb to sIgM to examine chemotactic activity of CKβ-11/MIP-3β/ELC on differentiated B cells because sIgM is expressed on differentiated B cells, but not on pro/pre-B cells, while CD19 is broadly expressed from pro/pre-B cells to differentiated B cells (27). CKβ-11/MIP-3β/ELC attracted $sIgM^+CD19^+$ B cells demonstrating its activity on these differentiated B cells (data not shown). It had been shown that CKβ-11/MIP-3β/ELC binds EBI1/BLR2/CCR7 (18) and EBI1/BLR2/CCR7-specific mRNA was detected in all EBV-positive B cell lines (28). It had been reported that the transcription of the *EBI1/BLR2/CCR7* gene was specifically induced in EBV-negative cells by estrogen-mediated activation of EBV nuclear Ag 2 (28). In this regard, we examined the chemotactic effect of CKβ-11/MIP-3β/ELC on an EBV-transformed B cell line, Priess. Consistent with reports on expression of EBI1/BLR2/CCR7 mRNA in EBV-transformed cell line (28), we observed that Priess cells were attracted to CKβ-11/MIP-3β/ELC in a dose-dependent fashion (Fig. 4*B*). Priess cells were quite motile by themselves, which resulted in a high background migration. This background level of migration into the lower chamber was decreased by adding CKβ-11/MIP-3β/ELC into the upper chamber forming a negative gradient of CKβ-11/MIP-3β/ELC (Fig. 4*B*).

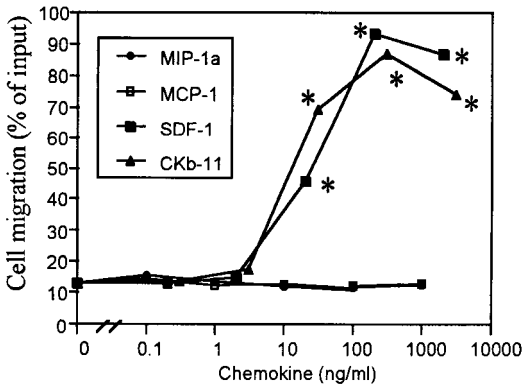

FIGURE 1. Chemotactic activity of CKβ-11/MIP-3β/ELC on $CD3^+$ T cells. Migration of $CD3^+$ T cells was represented as percentage of input $CD3^+$ T cells migrated into the lower chamber. * Indicates significant increase from control (background migration in medium), $p < 0.002$.

Monocytes from bone marrow and peripheral blood were not attracted to CKβ-11/MIP-3β/ELC, while MCP-1, used as a positive control, attracted peripheral blood (Fig. 4*C*) and bone marrow (Fig. 4*D*) monocytes. Primary chemoattractants for neutrophils are the CXC chemokines such as IL-8, GRO-α, -β, -γ, ENA78, and GCP-2, all of which have an ELR amino acid sequence motif N-terminal to the CXC amino acid sequence. CC chemokines and some CXC chemokines, such as SDF-1 that have no Glu-Leu-Arg (ELR) motif, have no chemotactic activity for neutrophils (3, 15, 29, 30). Like most other CC chemokines, CKβ-11/MIP-3β/ELC showed no chemotactic activity for granulocytes (Fig. 4*E*). Many chemokines are highly conserved across species. An extreme example is the SDF-1, which differs in only one amino acid between

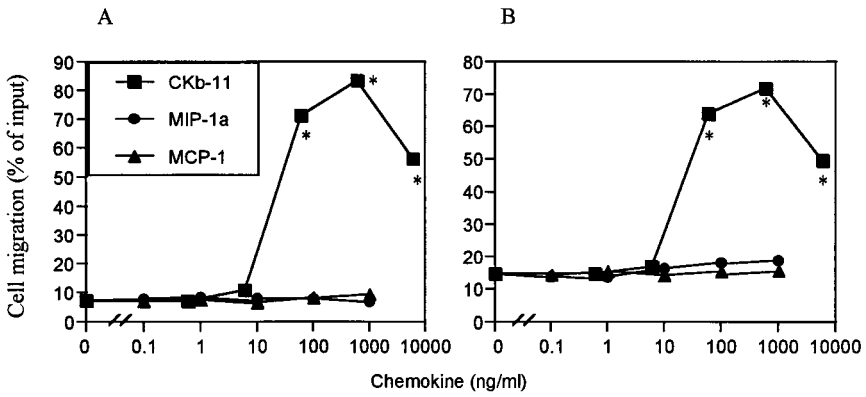

FIGURE 2. Effects of CKβ-11/MIP-3β/ELC on attraction of $CD4^+$ (*A*) and $CD8^+$ (*B*) T cell subsets. Indicated chemokines at various concentrations were added to the lower chamber to attract lymphocytes from the upper chamber. Migrated $CD4^+CD8^-$ and $CD4^-CD8^+$ T cell subsets were counted after 3 h by staining with fluorescent mAbs to CD4 and CD8 (PE conjugated for CD4 and TRI conjugated for CD8) followed by counting with FACscan for 20 s. Results are expressed as percentage of input cells. * Indicates significant increase from control (background migration in medium), $p < 0.01$.

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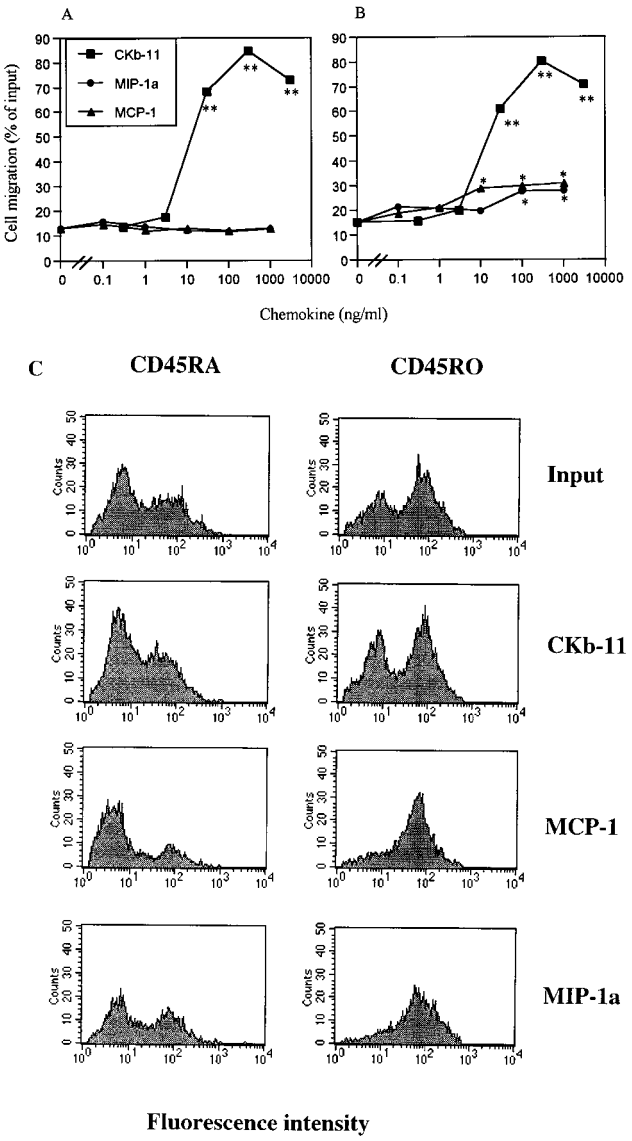

FIGURE 3. Effects of CKβ-11/MIP-3β/ELC on chemoattraction of $CD3^+CD45RA^+$ (*A*) and $CD3^+CD45RO^+$ (*B*) T cell subtypes. *, ** Indicate significant increase from control (background migration in medium); $*p < 0.05$, $**p < 0.002$. *C*, Phenotypic analysis of $CD3^+CD45RA^+$ or $CD3^+CD45RO^+$ T cells attracted to CKβ-11/MIP-3β/ELC, MIP-1α, MCP-1, and SDF-1 at their optimal concentration (200 ng/ml). Lymphocytes were added to the upper chamber and migrated cells were stained with anti-CD45RA, CD45RO, and CD3 mAbs. $CD3^+CD45RA^{+/-}$ or $CD3^+CD45RO^{+/-}$ T cells that migrated to each chemokine are shown.

mouse and human, and mouse SDF-1 is active on human leukocytes (15). Although the mouse counterpart of CKβ-11/MIP-3β/ELC has not been identified, human CKβ-11/MIP-3β/ELC attracted mouse splenocytes demonstrating its cross-species activity (Fig. 4*F*).

Pertussis toxin-sensitive CKβ-11/MIP-3β/ELC-dependent chemotaxis and actin polymerization by CKβ-11/MIP-3β/ELC

All chemokines use receptors with seven-transmembrane spanning domains, which are known to be coupled to trimeric G proteins. *Bordetella pertussis* toxin is known to inhibit signaling from a $G_{\alpha i}$ protein-coupled receptor (31). Pertussis toxin demonstrated dose response inhibition of chemotaxis in response to CKβ-11/MIP-3β/ELC (Fig. 5*A*) indicating that, like other chemokine receptors, CKβ-11/MIP-3β/ELC signaling for chemotaxis of cells is transmitted through heterotrimeric $G_{\alpha i}$ proteins, which are coupled to seven transmembrane-spanning chemokine receptors, most likely EBI1/BLR2/CCR7 (18, 28, 32, 33).

Many chemoattractants induce actin polymerization, which is involved in chemokine-dependent cell activation and cell migration. We observed the ability of CKβ-11/MIP-3β/ELC′ to induce actin polymerization in T cells (Fig. 5*B*). EC_{50} for actin polymerization was observed at about 30 ng/ml CKβ-11/MIP-3β/ELC and optimum effects were observed at concentrations ≥100 ng/ml. The starting point of the optimum concentration range for actin polymerization by CKβ-11/MIP-3β/ELC was similar to that for chemotaxis.

Calcium mobilization in lymphocytes by CKβ-11/MIP-3β/ELC

Chemokine binding to G protein-coupled seven transmembrane-spanning receptors induces calcium mobilization. It is known that phospholipase C β2 is involved in generation of inositol triphosphate upon IL-8 binding to its receptor, CXCR1, resulting in intracellular calcium release (34, 35). We observed that CKβ-11/MIP-3β/ELC induced calcium mobilization in lymphocytes. This was stronger and more prolonged than that induced by MIP-1α and RANTES at the same concentration (Fig. 6). Calcium mobilization by CKβ-11/MIP-3β/ELC was not desensitized by MIP-1α or RANTES. In addition, calcium mobilization by either MIP-1α or RANTES was not desensitized by CKβ-11/MIP-3β/ELC. This cross-desensitization experiment implies that CKβ-11/MIP-3β/ELC does not use the receptors for MIP-1α and RANTES, which have been reported to share receptors CCR1, CCR4, and CCR5, and supports the previous report that it does not bind these CC chemokine receptors (18). SDF-1 also induced calcium flux in lymphocytes (Fig. 6). SDF-1-pretreatment desensitized calcium mobilization by CKβ-11/MIP-3β/ELC completely, while CKβ-11/MIP-3β/ELC did not desensitize SDF-1-induced calcium mobilization.

Discussion

In this report, we describe functional characteristics of CKβ-11/MIP-3β/ELC such as identifying target cells for chemotactic activity, actin polymerization, and calcium mobilization. Purified recombinant CKβ-11/MIP-3β/ELC expressed in CHO cells showed efficacious chemotactic activity for $CD3^+$ T cells and $CD19^+$ B cells, but not for monocytes and granulocytes. Consistent with this finding, mRNA of EBI1/BLR2/CCR7, the receptor for CKβ-11/MIP-3β/ELC, is detected in T and B cell lines (32, 33), and EBI1/BLR2/CCR7 is the only known receptor for CKβ-11/MIP-3β/ELC (18). Primary amino acid structure of CKβ-11/MIP-3β/ELC showed a typical CC chemokine structure with four cysteine residues: two contiguous cysteine residues and two more separated down stream cysteine residues. However, CKβ-11/MIP-3β/ELC shows low amino acid similarity to other CC chemokines, Eotaxin-2 (24.7%) (36), MIP-1α (25.7%) (37), RANTES (29.4%) (38), and MIP-1β (29.0%) (39) (Table II). CKβ-11/MIP-3β/ELC forms an isolated subfamily of the CC chemokine superfamily with another chemokine, Exodus-1/CKβ-4 (40).

It appears that some chemokines have their own chemotactic specificity, spectrum, and potency for certain T cell subtypes. Efficacy of CKβ-11/MIP-3β/ELC in chemoattraction of T cells leads us to speculate that EBI1/BLR2/CCR7 would be expressed on most T cell subtypes. CKβ-11/MIP-3β/ELC was reported (18) as not binding promiscuously to many CC chemokine receptors such as CCR1 through CCR6 and other orphan receptors, V28/CMKBLR1 (41, 42), GPR-9-6 (GenBank accession no. U45982), and

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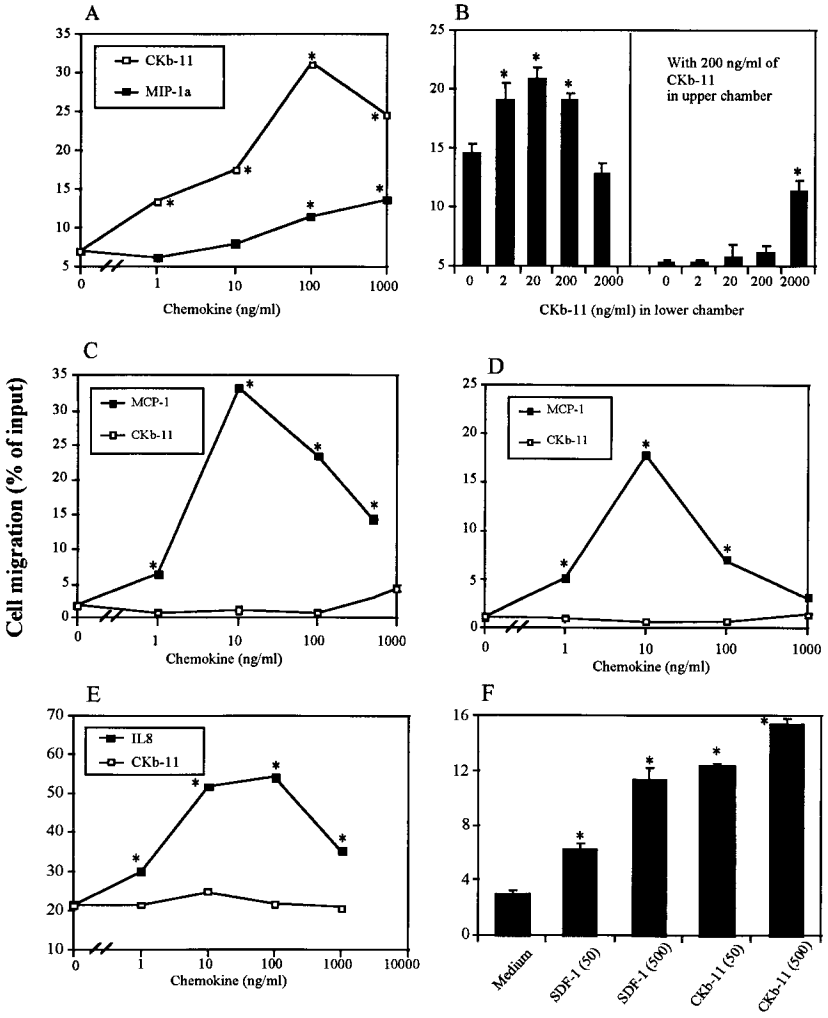

FIGURE 4. Effects of CKβ-11/MIP-3β/ELC on migration of human B cells, monocytes, granulocytes, and mouse splenocytes. *A*, Chemotactic effects of CKβ-11/MIP-3β/ELC on $CD3^{-}CD19^{+}$ B cells. Chemotactic effects of CKβ-11/MIP-3β/ELC on B cell line, Priess (*B*), peripheral blood monocytes (*C*), bone marrow monocytes (*D*), peripheral blood granulocytes (*E*), and mouse splenocytes (*F*). For *F*, chemokine concentrations, 50 and 500 ng/ml, were used and shown in parentheses. * Indicates significant increase from control (background migration in medium), $p < 0.05$. Error bars in *B* and *F* represent range of duplicates.

BLR1 (43). The mRNA of CKβ-11/MIP-3β/ELC was detected in the thymus and lymph nodes at a high level, and in the small intestine, colon, and lung at a lower level by others (18) and us (data not shown). Attraction of all T cell subtypes tested so far and constitutive expression of CKβ-11/MIP-3β/ELC mRNA in several organs suggests that CKβ-11/MIP-3β/ELC may be involved in diverse T cell trafficking involving T cell migration in thymus and maturation of T cells and T cell migration to lymph nodes, small intestine, colon, and lung. Since it attracts both T and B cells (Figs. 1, 2, 3, and 4, *A* and *B*) and is produced by activated macrophages (17), CKβ-11/MIP-3β/ELC may be important in colocalization of T and B cells in secondary lymphoid tissues for their interaction.

SDF-1 attracted about 10-fold more lymphocytes than other chemoattractants such as MCP-1, MIP-1α, IL-8, and RANTES (15). CKβ-11/MIP-3β/ELC shows a similar efficacy to the CXC chemokine, SDF-1, in attracting T cells. SDF-1 is a strong chemoattractant, but not a selective chemoattractant for subtypes of T cells in that it attracts CD45RA-, CD45RO-, CD4-, and CD8-expressing T cells (15). CKβ-11/MIP-3β/ELC was also not selective in attracting T cell subtypes except that it had a consistently slightly greater attraction for

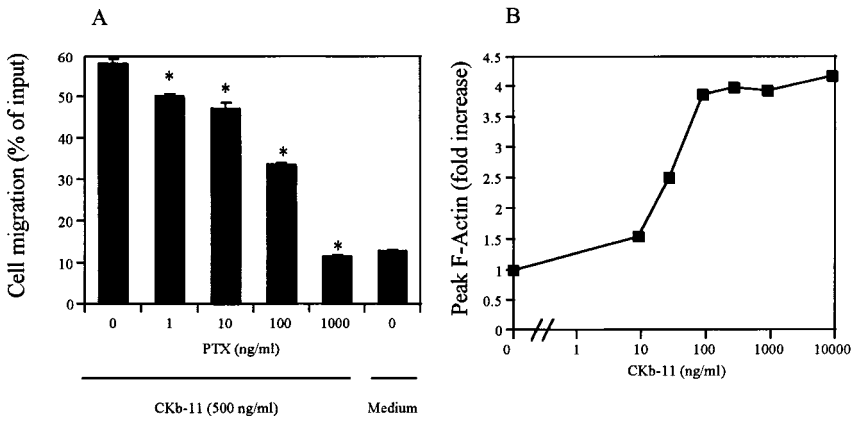

FIGURE 5. *A*, Sensitivity of CKβ-11/MIP-3β/ELC-induced T cell chemotaxis in response to *Bordetella pertussis* toxin. Lymphocytes were preincubated with pertussis toxin at the indicated concentrations for 45 min before start of the chemotaxis experiment. * Indicates significant increase from control (background migration in medium), $p < 0.05$. Error bars represent range of duplicates. *B*, Actin polymerization by CKβ-11/MIP-3β/ELC in lymphocytes.

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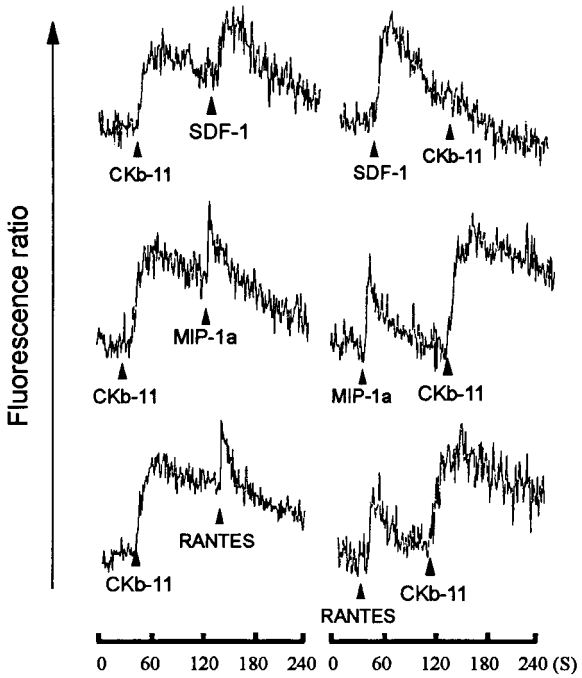

FIGURE 6. Calcium mobilization by CKβ-11/MIP-3β/ELC in lymphocytes. CKβ-11/MIP-3β/ELC, MIP-1α, RANTES, and SDF-1 were used at 50 nM. The *x*-axis and *y*-axis, respectively, represent time in seconds and ratio of fluorescence. Results are representative of four independent experiments using lymphocytes from four different donors.

$CD4^+$ than $CD8^+$ T cells. The chromosomal locations of human SDF-1 (44) and CKβ-11/MIP-3β/ELC (18) are 10 and 9, respectively, while most other CXC and CC chemokines are clustered, with a few exceptions, on chromosomes 4 and 17, respectively. Both SDF-1 and CKβ-11/MIP-3β/ELC are distantly related in primary amino acid sequence to other CC and CXC chemokines. The different chromosomal locations and DNA sequence structures of these two chemokine genes may suggest that these genes have duplicated and evolved earlier than many chemokine genes clustering on chromosomes 4 and 17. Both chemokines were potent in inducing calcium mobilization. SDF-1 desensitizes calcium mobilization by CKβ-11/MIP-3β/ELC, while CKβ-11/MIP-3β/ELC does not desensitize SDF-1-dependent calcium mobilization showing a dominance of SDF-1 over CKβ-11/MIP-3β/ELC in calcium mobilization in lymphocytes. This is unusual in that SDF-1 is a CXC chemokine whereas CKβ-11/MIP-3β/ELC is a CC chemokine. This could be due to two possibilities: 1) SDF-1 may bind EBI1/BLR2/CCR7, whereas CKβ-11/MIP-3β/ELC may not bind CXCR4, the receptor for SDF-1; or 2) heterologous cross-desensitization between CXCR4 and EBI1/BLR2/CCR7 may exist. This latter possibility has recently been demonstrated for the IL-8, C5a, and FMLP receptors on human neutrophils (45). Two potent T cell chemoattractants may chemotactically interact with each other to regulate T cell trafficking by cooperating in attraction and desensitizing the other's signaling.

Table II. *Amino acid similarity and divergence of CKβ-11/MIP-3β/ELC to selected CC chemokines*[a]

	CKβ-11	Eotaxin-2	MIP-1α	MIP-1β	RANTES
CKβ-11		24.7	25.7	29.0	29.4
Eotaxin-2	70.8		35.7	30.4	22.1
MIP-1α	70.1	61.4		68.1	45.6
MIP-1β	65.2	66.7	31.9		48.5
RANTES	69.2	73.5	52.9	48.5	

[a] Percent similarity is in upper triangle and percent divergence is in lower triangle. Amino acid similarity vs divergence was determined by the clustal method using Megalign PAM 250 by DNASTAR (Madison, WI) according to the definitions and method described by Dagoff (46).

Acknowledgments

We thank Donna Cusimono for vector construction, Joyce Mao and Stephanie Van Horn for oligonucleotide synthesis and DNA sequencing, Laura Grayson for CHO cell transfection and growth, Gil Scott for purification of CKβ-11, Dean McNulty for analytical characterization of CKβ-11, and Rebecca Miller and Cindy Booth for secretarial assistance.

References

1. Negus, R. P. 1996. The chemokines: cytokines that direct leukocyte migration. *J. R. Soc. Med. 89:312.*
2. Premack, B. A., and T. J. Schall. 1996. Chemokine receptors: gateways to inflammation and infection. *Nat. Med. 2:1174.*
3. Schall, T. J., and K. B. Bacon. 1994. Chemokines, leukocyte trafficking, and inflammation. *Curr. Opin. Immunol. 6:865.*
4. Strieter, R. M., T. J. Standiford, G. B. Huffnagle, L. M. Colletti, N. W. Lukacs, and S. L. Kunkel. 1996. "The good, the bad, and the ugly": the role of chemokines in models of human disease. *J. Immunol. 156:3583.*
5. Kelner, G. S., J. Kennedy, and K. B. Bacon. 1994. Lymphotactin: a cytokine that represents a new class of chemokine. *Science 266:1395.*
6. Bazan, J. F., K. B. Bacon, G. Hardiman, W. Wang, K. Soo, D. Rossi, D. R. Greaves, A. Zlotnik, and T. J. Schall. 1997. A new class of membrane-bound chemokine with a CX3C motif. *Nature 385:640.*
7. Schall, T. 1997. Fractalkine—a strange attractor in the chemokine landscape. *Immunol. Today 18:147.*
8. Nomiyama, H., T. Imai, J. Kusuda, R. Miura, D. F. Callen, and O. Yoshie. 1997. Assignment of the human CC chemokine gene TARC (SCYA17) to chromosome 16q13. *Genomics 40:211.*
9. Hieshima, K., T. Imai, G. Opdenakker, J. V. Damme, J. Kusuda, H. Tei, Y. Sakaki, K. Takatsuki, R. Miura, O. Yoshie, and H. Nomiyama. 1997. Molecular cloning of a novel human CC chemokine liver and activation-regulated chemokine (LARC) expressed in liver: chemotactic activity for lymphocytes and gene localization on chromosome 2. *J. Biol. Chem. 272:5846.*
10. Gimbrone, M. A., Jr., M. S. Obin, A. F. Brock, E. A. Luis, P. E. Hass, C. A. Hebert, Y. K. Yip, D. W. Leung, D. G. Lowe, W. J. Kohr, W. C. Darbonne, K. B. Bechtol, and J. B. Baker. 1989. Endothelial interleukin-8: a novel inhibitor of leukocyte-endothelial interactions. *Science 246:1601.*
11. Tanaka, Y., D. H. Adams, S. Hubscher, H. Hirano, U. Siebenlist, and S. Shaw. 1993. T-cell adhesion induced by proteoglycan-immobilized cytokine MIP-1 β. *Nature 361:79.*
12. Taub, D. D., A. R. Lloyd, K. Conlon, J. M. Wang, J. R. Ortaldo, A. Harada, K. Matsushima, D. J. Kelvin, and J. J. Oppenheim. 1993. Recombinant human interferon-inducible protein 10 is a chemoattractant for human monocytes and T lymphocytes and promotes T cell adhesion to endothelial cells. *J. Exp. Med. 177:1809.*
13. Luscinskas, F. W., J. M. Kiely, H. Ding, M. S. Obin, C. A. Hebert, J. B. Baker, and M. A. Gimbrone, Jr. 1992. In vitro inhibitory effect of IL-8 and other chemoattractants on neutrophil-endothelial adhesive interactions. *J. Immunol. 149:2163.*
14. Aiuti, A., I. J. Webb, C. Bleul, T. Springer, and J. C. Gutierrez-Ramos. 1997. The chemokine SDF-1 is a chemoattractant for human $CD34^+$ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of $CD34^+$ progenitors to peripheral blood. *J. Exp. Med. 185:111.*
15. Bleul, C. C., R. C. Fuhlbrigge, J. M. Casanovas, A. Aiuti, and T. A. Springer. 1996. A highly efficacious lymphocyte chemoattractant, stromal cell-derived factor 1 (SDF-1). *J. Exp. Med. 184:1101.*
16. Nagasawa, T., H. Kikutani, and T. Kishimoto. 1994. Molecular cloning and structure of a pre-B-cell growth-stimulating factor. *Proc. Natl. Acad. Sci. USA 91:2305.*
17. Rossi, D. L., A. P. Vicari, K. Franz-Bacon, T. K. McClanahan, and A. Zlotnik. 1997. Identification through bioinformatics of two new macrophage proinflammatory human chemokines: MIP-3 alpha and MIP-3 beta. *J. Immunol. 158:1033.*
18. Yoshida, R., T. Imai, K. Hieshima, J. Kusuda, M. Baba, M. Kitaura, M. Nishimura, M. Kakizaki, H. Nomiyama, and O. Yoshie. 1997. Molecular cloning of a novel human CC chemokine EBI1-ligand chemokine that is a specific functional ligand for EBI1, CCR7. *J. Biol. Chem. 272:13803.*
19. Aiyar, N., E. Baker, H. L. Wu, P. Nambi, R. M. Edwards, J. J. Trill, C. Ellis, and D. J. Bergsma. 1994. Human AT1 receptor is a single copy gene: characterization in a stable cell line. *Mol. Cell. Biochem. 131:75.*
20. Hensley, P., P. J. McDevitt, I. Brooks, J. J. Trill, J. A. Feild, D. E. McNulty, J. R. Connor, D. E. Griswold, N. V. Kumar, K. D. Kopple, S. A. Carr, B. J. Dalton, and K. Johanson. 1994. The soluble form of E-selectin is an asymmetric monomer. *J. Biol. Chem. 269:23946.*
21. Berkhout, T. A., H. Sarau, K. Moores, J. R. White, N. Elshourbagy, E. Appelbaum, T. J. Reape, M. Brawner, J. Makwana, J. J. Foley, D. B. Schmidt, C. Imburgia, D. McNulty, J. Matthews, K. O'Donnell, D. O'Shannessy, M. Scott,

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P. H. E. Groot, and C. Macphee. 1997. 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.*

22. Zigmond, S. H., and J. G. Hirsch. 1973. Leukocyte locomotion and chemotaxis: new methods for evaluation, and demonstration of a cell-derived chemotactic factor. *J. Exp. Med. 137:387.*
23. Qin, S., G. LaRosa, J. J. Campbell, H. Smith-Heath, N. Kassam, X. Shi, L. Zeng, E. C. Buthcher, and C. R. Mackay. 1996. Expression of monocyte chemoattractant protein-1 and interleukin-8 receptors on subsets of T cells: correlation with transendothelial chemotactic potential. *Eur. J. Immunol. 3:640.*
24. Schall, T. J., K. Bacon, K. J. Toy, and D. V. Goeddel. 1990. Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. *Nature 347:669.*
25. Jinquan, T., B. Moller, M. Storgaard, N. Mukaida, J. Bonde, N. Grunnet, F. T. Black, C. G. Larsen, K. Matsushima, and K. Thestrup-Pedersen. 1997. Chemotaxis and IL-8 receptor expression in B cells from normal and HIV-infected subjects. *J. Immunol. 158:475.*
26. Schall, T. J., K. Bacon, R. D. Camp, J. W. Kaspari, and D. V. Goeddel. 1993. Human macrophage inflammatory protein alpha (MIP-1 alpha) and MIP-1 beta chemokines attract distinct populations of lymphocytes. *J. Exp. Med. 177:1821.*
27. Loken, M. R., V. O. Shah, K. L. Dattilio, and C. I. Civin. 1987. Flow cytometric analysis of human bone marrow. II. Normal B lymphocyte development. *Blood 70:1316.*
28. Burgstahler, R., B. Kempkes, K. Steube, and M. Lipp. 1995. Expression of the chemokine receptor BLR2/EBI1 is specifically transactivated by Epstein-Barr virus nuclear antigen 2. *Biochem. Biophys. Res. Commun. 215:737.*
29. Baggiolini, M., B. Dewald, and B. Moser. 1994. Interleukin-8 and related chemotactic cytokines-CXC and CC chemokines. *Adv. Immunol. 55:97.*
30. Murphy, P. M. 1994. The molecular biology of leukocyte chemoattractant receptors. *Annu. Rev. Immunol. 12:593.*
31. Katz, A., D. Wu, and M. I. Simon. 1992. Subunits beta gamma of heterotrimeric G protein activate beta 2 isoform of phospholipase C. *Nature 360:686.*
32. Birkenbach, M., K. Josefsen, R. Yalamanchili, G. Lenoir, and E. Kieff. 1993. Epstein-Barr virus-induced genes: first lymphocyte-specific G protein-coupled peptide receptors. *J. Virol. 67:2209.*
33. Schweickart, V. L., R. Godiska, M. G. Byers, R. L. Eddy, Jr, T. B. Shows, and P. W. Gray. 1994. Cloning of human and mouse EBI1, a lymphoid-specific G-protein-coupled receptor encoded on human chromosome 17q12-q21.2. *Genomics 23:643.*
34. Bokoch, G. M. 1995. Chemoattractant signaling and leukocyte activation. *Blood 86:1649.*
35. Wu, D., G. J. LaRosa, and M. I. Simon. 1993. G protein-coupled signal transduction pathways for interleukin-8. *Science 262:101.*
36. Forssmann, U., M. Uguccioni, P. Loetscher, C. A. Dahinden, H. Langen, M. Thelen, and M. Baggiolini. 1997. Eotaxin-2, a novel CC chemokine that is selective for the chemokine receptor CCR3, and acts like eotaxin on human eosinophil and basophil leukocytes. *J. Exp. Med. 185:2171.*
37. Obaru, K., T. Hattori, Y. Yamamura, K. Takatsuki, H. Nomiyama, S. Maeda, and K. Shimada. 1989. A cDNA clone inducible in human tonsillar lymphocytes by a tumor promoter codes for a novel protein of the beta-thromboglobulin superfamily. *Mol. Immunol. 26:423.*
38. Schall, T. J., J. Jongstra, B. J. Dyer, J. Jorgensen, C. Clayberger, M. M. Davis, and A. M. Krensky. 1988. A human T cell-specific molecule is a member of a new gene family. *J. Immunol. 141:1018.*
39. Sherry, B., P. Tekamp-Olson, C. Gallegos, D. Bauer, G. Davatelis, S. D. Wolpe, F. Masiarz, D. Coit, and A. Cerami. 1988. Resolution of the two components of macrophage inflammatory protein 1, and cloning and characterization of one of those components, macrophage inflammatory protein 1 beta. *J. Exp. Med. 168:2251.*
40. Baggiolini, M., B. Dewald, and B. Moser. 1997. Human chemokines: an update. *Annu. Rev. Immunol. 15:675.*
41. Raport, C. J., V. L. Schweickart, R. L. Eddy, Jr., T. B. Shows, and P. W. Gray. 1995. The orphan G-protein-coupled receptor-encoding gene V28 is closely related to genes for chemokine receptors and is expressed in lymphoid and neural tissues. *Gene 163:295.*
42. Combadiere, C., S. K. Ahuja, and P. M. Murphy. 1995. Cloning, chromosomal localization, and RNA expression of a human beta chemokine receptor-like gene. *DNA Cell Biol. 14:673.*
43. Dobner, T., I. Wolf, T. Emrich, and M. Lipp. 1992. Differentiation-specific expression of a novel G protein-coupled receptor from Burkitt's lymphoma. *Eur. J. Immunol. 22:2795.*
44. Shirozu, M., T. Nakao, J. Inazawa, K. Tashiro, H. Tada, T. Shinohara, and T. Honjo. 1995. Structure and chromosomal location of the human stromal cell-derived factor 1 (*SDF1*) gene. *Genomics 28:495.*
45. Sabroe, I., T. J. Williams, C. A. Hébert, and P. D. Collins. 1997. Chemoattractant cross-desensitization of the human neutrophil IL-8 receptor involves receptor internalization and differential receptor subtype regulation. *J. Immunol. 158:1361.*
46. Dagoff, M. O. 1978. A model of evolutionary change in proteins: matrices for detecting distant relationships. In *Atlas of Protein Sequences and Structure*. Vol. 15, Suppl. 3. M. O. Dagoff, ed. National Biomedical Research Foundation, Washington DC, pp. 345–358.
47. Kim, C. H., and H. E. Broxmeyer. 1998. In vitro behavior of hematopoietic progenitor cells under the influence of chemoattractants: SDF-1, steel factor and the bone marrow environment. *Blood 91:100.*

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