Molecular Cloning of a Novel Human CC Chemokine EBI1-ligand Chemokine That Is a Specific Functional Ligand for EBI1, CCR7*

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By searching the expressed sequence tag (EST) database, we identified partial cDNA sequences encoding a novel human CC chemokine. We determined the complete cDNA sequence that encodes a highly basic polypeptide of a total 98 amino acids with 20 to 30% identity to other human CC chemokines. We termed this novel chemokine from EBI1-Ligand Chemokine as ELC (see below). The ELC mRNA was most strongly expressed in the thymus and lymph nodes. Recombinant ELC protein was expressed as a fusion protein with the Flag tag (ELC-Flag). For receptor-binding assays, recombinant ELC protein fused with the secreted form of alkaline phosphatase (SEAP) was used. By stably expressing five CC chemokine receptors (CCR1 to 5) and five orphan receptors, ELC-SEAP was found to bind specifically to an orphan receptor EBI1. Only ELC-Flag, but not MCP-1, MCP-2, MCP-3, eotaxin, MIP-1α, MIP-1β, RANTES (regulated on activation normal T cell expressed and secreted), thymus and activation-regulated chemokine (TARC), or liver and activation-regulated chemokine (LARC), competed with ELC-SEAP for EBI1. ELC-Flag-induced transient calcium mobilization and chemotactic responses in EBI1-transfected cells. ELC-Flag also induced chemotaxis in HUT78 cells expressing endogenous EBI1 at high levels. By somatic hybrid and radiation hybrid analyses, the gene for ELC (SCYA19) was mapped to chromosome 9p13 instead of chromosome 17q11.2 where the genes for CC chemokines are clustered. Taken together, ELC is a highly specific ligand for EBI1, which is known to be expressed in activated B and T lymphocytes and strongly up-regulated in B cells infected with Epstein-Barr virus and T cells infected with herpesvirus 6 or 7. ELC and EBI1 may thus play roles in migration and homing of normal lymphocytes, as well as in pathophysiology of lymphocytes infected with these herpesviruses. We propose EBI1 to be designated as CCR7.

The chemokines are a group of approximately 70–90 amino acid structurally related polypeptides that play important roles in inflammatory and immunological responses primarily by virtue of their ability to recruit selective leukocyte subsets (1, 2). Some chemokines may also play roles in normal lymphocyte recirculation and homing (3, 4). Furthermore, certain chemokines have been shown to have other biological activities such as suppression of hematopoiesis (5–7), stimulation of angiogenesis (8), suppression of angiogenesis (9, 10), suppression of apoptosis (11), and suppression of human immunodeficiency virus infection (12–14). The chemokines are grouped into the CXC and CC subfamilies on the basis of the arrangement of the two NH₂-terminal cysteine residues. One amino acid separates the two cysteine residues in the CXC chemokines, whereas the two cysteines are adjacent in the CC chemokines. Most CXC chemokines are potent attractants for neutrophils, whereas most CC chemokines are able to recruit monocytes, and also lymphocytes, basophils, and/or eosinophils with variable selectivity (1, 2). Recently, a novel chemokine-like cytokine lympho-tactin/SCM-1 has been reported, which carries only the second and the fourth of the four cysteine residues conserved in the chemokines and seems to act specifically on lymphocytes (15, 16). This may suggest the existence of the C-type chemokine subfamily.

The specific effects of chemokines are mediated by a family of 7-transmembrane G-protein coupled receptors (17, 18). In humans, four CXC chemokine receptors (CXCR1 to 4) and five CC chemokine receptors (CCR1 to 5) have been defined for their ligand specificity: CXCR1 for IL-8 (19); CXCR2 for IL-8 and other CXC chemokines with the ELR motif (20–22); CXCR3 for IP-10 and MIG (23); CXCR4 for SDF-1/PBSF (13, 14); CCR1 for MIP-1α, RANTES and MCP-3 (24–27); CCR2 for MCP-1 and MCP-3 (27–29); CCR3 for eotaxin, RANTES, MCP-3 and MCP-4 (30–33); CCR4 for TARC (34); CCR5 for RANTES, MIP-1α, and MIP-1β (35, 36). Furthermore, there are a growing number of putative chemokine receptors whose ligands remain to be identified. In this regard, we have recently demonstrated that an orphan receptor GPR-CY42/DRY6/CCR-L3 (37) is the specific receptor for a novel human CC chemokine LARC (38) and, thus, have proposed CCR6 for its designation (39). Among the known orphan receptors, EBI1, being desig-

1 The abbreviations and other trivial names used are: SCM, single C motif; G-protein, heterotrimeric guanine nucleotide-binding regulatory protein; CXCR, CXC chemokine receptor; CCR, CC chemokine receptor; IL-8, interleukin 8; IP-10, interferon-γ inducible protein 10; MIG, monokine induced by interferon-γ; SDF, stroma-derived factor; PBSF, pre-B cell stimulatory factor; MIP, macrophage inflammatory protein; RANTES, regulated on activation normal T cell expressed and secreted; MCP, monocyte chemotactic protein; TARC, thymus and activation-regulated chemokine; LARC, liver and activation-regulated chemokine; EBI1, EBV-induced gene; HHV, human herpesvirus; EST, expressed sequence tag; RACE, rapid amplification of cDNA end; PCR, polymerase chain reaction; BSA, bovine serum albumin; BLYR, Burkitt’s lymphoma receptor; ELC, EBI1-ligand chemokine; SEAP, secreted form of alkaline phosphatase; EBV, Epstein-Barr virus; EBNA, EBV-encoded nuclear antigen; PBS, phosphate-buffered saline.

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nated from Epstein-Barr virus (EBV)-induced gene 1 (40), is expressed in various lymphoid tissues and activates B and T lymphocytes (40, 41). EB1 is notable because it is strongly up-regulated in B cells upon infection with EBV (40, 42), is transactivated by EBV-encoded nuclear antigen 2 (EBNA-2) (42) and is also up-regulated in CD4+ T cells upon infection with human herpesvirus 6 (HHV-6) and HHV-7 (43).

The expressed sequence tags (ESTs) consist of partial “single pass” cDNA sequences from various tissues (44). Analysis of the EST data bases is becoming a powerful approach to look for new members of gene families. Recently, we have identified a number of novel human CC chemokines by initially searching the EST data bases for homology with known CC chemokine members (38, 45). Here we report a novel human CC chemokine that is expressed in various lymphoid tissues and turns out to be a specific high-affinity functional ligand for EB1 (40). Thus, we have designated this novel CC chemokine ELC from EB1-ligand chemokine. The ELC gene is mapped to chromosome 9p13 instead of 17q11.2 where the genes for most other CC chemokines are clustered. We now propose EB1 to be designated as CCR7.

EXPERIMENTAL PROCEDURES

Cells—Human hematopoietic cell lines were maintained in RPMI 1640 supplemented with 10% fetal calf serum. 293/EBNA-1 cells were purchased from Invitrogen (San Diego, CA) and maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. K562 cells and 293/EBNA-1 cells stably expressing CCR1 (24–27), CCR2B (27–29), CCR3 (30–33), CCR4 (35, 36), V28/CMK-1 (47, 48), GPR-9–64 (GenBankTM accession number U45982), EBI1 (40), and Burkitt’s lymphoma receptor 1 (BLR1) (49) were described previously (34).

EST Data Base Search—The dbEST (44) was searched with various human CC chemokine nucleotide sequences or amino acid sequences as queries using the data base search and analysis service Search Launcher (50) available on the World Wide Web. The program used was Basic Local Alignment Search Tool (51).

Isolation and Sequence of ELC cDNA—The full-length cDNA sequence was obtained by the rapid amplification of cDNA ends (RACE) method (52). In brief, 5’ and 3’ RACE polymerase chain reactions (PCR) were carried out using human fetal lung cDNA commercially available for RACE-PCR (CLONTECH, Palo Alto, CA). The cDNA was amplified by PCR with one of the gene-specific primers based on an EST sequence (GenBankTM accession number NT11167) (53). RACE-primer, GAGTGAAGGTTGGCTCTGCT; 3’ RACE-primer, GAGGTCAGTCTAGGTCAACAGCT and an AP1 primer (CLONTECH), which is complementary to part of the cDNA adaptor ligated at both ends of the cDNA. PCR was performed in a 50-μl reaction mixture containing 0.2 mM each of dNTPs, 10 pmol of each of the primers, 2.5 units of TAKARA LA Taq (Takara, Kyoto, Japan), 1× buffer supplied with the polymerase, and 0.55 μg of TaqStart antibody (CLONTECH). The PCR conditions were 5 cycles of 94 °C for 30 s and 72 °C for 4 min, 5 cycles of 94 °C for 30 s and 70 °C for 4 min, and then 25 cycles of 94 °C for 30 s and 68 °C for 4 min. The amplification products were cloned into pCR-II vector (Stratagene, La Jolla, CA) by T-A ligation and sequenced on both strands using gene-specific and commercial primers.

Northern Blot Analysis—This was carried out as described previously (53). In brief, multiple tissue blots, and immune blots were probed for 30 s and 70 °C for 4 min, and then 25 cycles of 94 °C for 30 s and 68 °C for 4 min. The amplification products were cloned into pCR-II vector (Stratagene, La Jolla, CA) by T-A ligation and sequenced on both strands using gene-specific and commercial primers.

Production and Purification of ELC Fusion Protein—ELC was expressed as a fusion protein with the secreted form of alkaline phosphatase (SEAP) with a COOH terminal tag of 6 histidine residue, the (His)6 tag, as described previously (38). In brief, the ELC cDNA was subcloned into pRDE-SEAP(His)6-Hyg (38) so that ELC was fused through a 5 amino acid linker (Ser-Arg-Ser-Ser-Gly) to SEAP with the (His)6 tag. To produce the ELC-SEAP fusion protein, 293/EBNA-1 cells (Invitrogen) were transfected with pRDE-ELC-SEAP(His)6-Hyg by using Lipofectamine (Life Technologies, Inc.). After 3–4 days, the culture supernatants were collected by centrifugation, filtered (0.22 μm), and added to 20 mM HEPES, pH 7.4, and 0.02% sodium azide. For NH2-terminal sequence analysis, the fusion protein was affinity purified by nickel-agarose chromatography (QIAGEN, Hilden, Germany). The concentration of ELC-SEAP was determined by a sandwich-type enzyme-linked immunosorbent assay as previously described (38). Briefly, 96-well microtiter plates (Maxisorb, Nunc, Roskilde, Denmark) were coated with 2 μg/ml of monoclonal anti-platelet alkaline phosphatase antibody (Medix Biotech, Foster City, CA) in 50 mM Tris-Cl, pH 9.5. After blocking nonspecific binding sites with 1% bovine serum albumin (BSA) in PBS, the samples were titrated in PBS with 0.02% Tween-20. After incubation for 1 h at room temperature, the plates were washed, incubated with biotinylated rabbit anti-placental alkaline phosphatase antibody diluted 1:500 for 1 h at room temperature, washed again, and incubated for 30 min with peroxidase-conjugated streptavidin (Vector Laboratories, Burlingam, CA). After washing, bound peroxidase was detected by 3,3′,5,5′-tetramethylbenzidine. The reaction was stopped by adding H2SO4, and the absorbance at 450 nm was read. The enzyme activity of SEAP and ELC-SEAP were determined by a chemiluminescence assay using the Great Escape Detection kit (CLONTECH). Purified placental alkaline phosphatase (Cosmo Bio, Tokyo, Japan) was used to generate the standard curve. Alkaline phosphatase activity was determined by the BCA kit (Pierce, Rockford, IL). NH2-terminal sequence analysis was performed on a protein sequencer (Shimazu, Tokyo, Japan).

Production of ELC-SEAP Fusion Protein—ELC was expressed as a fusion protein with the secreted form of alkaline phosphatase (SEAP) with a COOH terminal tag of 6 histidine residue, the (His)6 tag, as described previously (38). In brief, the ELC cDNA was subcloned into pRDE-SEAP(His)6-Hyg (38) so that ELC was fused through a 5 amino acid linker (Ser-Arg-Ser-Gly) to SEAP with the (His)6 tag. To produce the ELC-SEAP fusion protein, 293/EBNA-1 cells (Invitrogen) were transfected with pRDE-ELC-SEAP(His)6-Hyg by using Lipofectamine (Life Technologies, Inc.). After 3–4 days, the culture supernatants were collected by centrifugation, filtered (0.22 μm), and added to 20 mM HEPES, pH 7.4, and 0.02% sodium azide. For NH2-terminal sequence analysis, the fusion protein was affinity purified by nickel-agarose chromatography (QIAGEN, Hilden, Germany). The concentration of ELC-SEAP was determined by a sandwich-type enzyme-linked immunosorbent assay as previously described (38).

Binding Assay—This was carried out as described previously (38). In brief, 2 × 106 cells were incubated for 1 h at 16 °C with 1 μM of SEAP or ELC-SEAP without or with increasing concentrations of unlabeled chemokines in 200 μl of RPMI 1640 containing 20 mM HEPES, pH 7.4, 1% BSA, and 0.02% sodium azide. MCP-1, eotaxin, LARC, and TARC were prepared as described previously (30, 38, 53). MIP-1α, MIP-1β, MCP-2, MCP-3, and RANTES were purchased from Pepro Tech (Rocky Hill, NJ). After that, cells were washed 5 times and lysed. ELISA 50 μl of 10 mM Tris-HCl, pH 8.0, and 1% Triton X-100. Samples were heated at 65 °C for 10 min to inactivate cellular phosphatases and centrifuged to remove cell debris. AP activity in μl of lysate was determined by the chemiluminescence assay as described above. All samples were determined in duplicate. The binding data were analyzed by the LIGAND program (55).

* Deposited by L. L. Lautens, H. L. Tiffany, J.-L. Guo, W. Modis, P. M. Murphy, and T. I. Bonner with accession number U45982.
Calcium Mobilization Assay—K562 cells stably expressing cloned chemokine receptors were suspended at 3 × 10^6 cells/ml in Hank’s balanced salt solution supplemented with 1 mg/ml BSA and 10 mM HEPES, pH 7.4, and loaded with 1 μM Fura-PE3-AM (Texas Fluorescence Labs) by incubation for 1 h at room temperature in the dark. Loaded cells were washed twice with Hank’s balanced salt solution-BSA and resuspended in the same buffer at 2.5 × 10^6 cells/ml. To measure intracellular calcium, 2 μl of the cell suspension was placed in a quartz cuvette in a Perkin-Elmer LS 50B spectrofluorimeter and stimulated with chemokines at 37 °C. Fluorescence was monitored at 340 nm (l_ex1), 380 nm (l_ex2), and 510 nm (l_em) every 200 ms. To determine EC_{90} for calcium mobilization, a dose-response curve was generated in each experiment by plotting percent maximum responses.

Migration Assay—The cell migration assay was performed using a 48-well microchemotaxis chamber as described previously (53). In brief, chemokines were diluted in Hepes-buffered RPMI 1640 supplemented with 1% BSA and placed in lower wells (30 μl/well). Cells suspended in RPMI 1640, 1% BSA at 2 × 10^6/ml (293/EBNA-1 cells) or at 8 × 10^5/ml (HUVEC) were added to upper wells (50 μl well) that were separated from lower wells by a polyvinylpyrrolidone-free polycarbonate filter with 5- or 8-μm pores precoated with type IV collagen. The chamber was incubated for 2 or 4 h at 37 °C in 5% CO2, 95% air. Filters were removed and stained with Diff-Quik (Harleco, Gibbstown, NJ). Migration assay was divided into five randomly selected high-power fields (×400) per well. All assays were done in triplicate.

Somatic Cell and Radiation Hybrid Mapping—DNAs of the human × rodent somatic cell hybrids containing human monoclonosomes (National Institute of General Medical Science Mapping Panel No. 2, Version 2, Coriell Cell Repositories, Camden, NJ) and of 93 radiation hybrids (56) (Gene Bridge 4 Mapping Panel, Research Genetics, Huntsville, AL) were analyzed by PCR using ELC primers (5'-GAGCCCCGAGTCGAGCTAAGCRTT and 5'-CTCTGACCACACTCACCCCCTCTGGC). The PCR conditions were 5 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min in a 25-μl reaction buffer containing 0.25 mM each of dNTP, 1 pmol each of the primers, and 1.25 units of AmpliTaq Gold (Perkin-Elmer, Norwalk, CT). PCR products were electrophoresed on 2% agarose gel. The product was 166-base pairs in length. Radiation hybrid mapping data were analyzed by accessing the server at http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl.

RESULTS

Cloning of ELC cDNA—By searching the EST data base (44) with nucleotide and amino acid sequences of various CC chemokines, we identified seven EST sequences potentially encoding a novel human CC chemokine (GenBank accession numbers T97450, D31180, D31431, W06519, W07401, N71167, and N80273) (Fig. 1). Later, we came to designate this novel CC chemokine ELC from EB11-ligand chemokine (see below), but we use this term hereinafter for the sake of convenience. To determine the full-length cDNA sequence, we carried out 5' and 3' RACE (52). The primers were designed from the EST sequence N71167 (Fig. 1). Since most ESTs were derived from fetal lung cDNA libraries, human fetal lung cDNA commercially prepared for RACE-PCR (CLONTECH) was used for the reaction. The full-length cDNA is 687 base pairs in length and contains a long open reading frame starting from the first methionine codon and encoding a highly basic polypeptide of a total 88 amino acids with a calculated molecular weight of 9,992 (Fig. 2A). The nucleotide sequence around the first methionine codon conforms well to the consensus sequence of the eukaryotic translational initiation site (57). The 3' noncoding region contains a typical ATTAAA polyadenylation signal but not the ATTTA motif for rapid mRNA degradation that is frequently found in the 3' noncoding regions of cytokines and chemokines (58).

The deduced polypeptide sequence contains a highly hydrophobic signal terminal region characteristic of a signal peptide with a putative cleavage site between Ser-21 and Gly-22 (Fig. 2A) (59). The predicted mature protein of 77 amino acids has a molecular weight of 8,800 and an isoelectric point of 10.11. There is no potential N-glycosylation site. The predicted mature protein shows significant homology to other human CC chemokines (58). The mature protein shows significant homology to other human CC chemokines (58).

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A Novel EBI1-Ligand Chemokine ELC

...arrangement. In addition, several other amino acid residues such as Phe-62, Val-79, and Leu-86 that are conserved in all other CC chemokines are present. The amino acid identity of the mature protein is 31% with MIP-1β (60), 30% with RANTES (61) and LARC (38), 28% with MIP-1α/LD78α (62), 26% with TARC (53), 23% with MCP-2 (63), MCP-3 (64) and I309 (65), and 21% with MCP-1 (66, 67) (Fig. 2B). Thus, the cDNA encodes a novel member of the CC chemokine subfamily.

Expression of ELC mRNA in Human Tissues—We determined the expression of mRNA in various human tissues (Fig. 3). The mRNA was found to be constitutively expressed at high levels in thymus and lymph nodes, at intermediate levels in colon and trachea, and at low levels in spleen, small intestine, lung, kidney, and stomach. Among lymphoid tissues, the mRNA was expressed at high levels in lymph nodes, thymus, and appendix. Spleen also contained the mRNA at low levels, but peripheral blood leukocytes, bone marrow, and fetal liver were virtually negative.

Production of Recombinant ELC Protein—To obtain ELC protein, we first tried the baculovirus expression system that we successfully employed for TARC and LARC (38, 53). But we were unable to get the ELC protein secreted from the cells, probably because the signal sequence of ELC was not recognized in insect cells (not shown). Previously, we found no adverse effect of the COOH-terminal Flag tag (54) on the activity of MCP-1 in comparison with non-tagged MCP-1 (data not shown). So we decided to produce the ELC protein tagged with the Flag sequence in the COOH terminus. 293/EBNA-1 cells were transfected with pDREF-ELC-Flag vector, and the ELC-Flag fusion protein in the culture supernatants was purified by anti-Flag affinity chromatography and reverse-phase high performance liquid chromatography. Recombinant ELC-Flag was eluted from the reverse-phase column as a single peak (Fig. 3A). When analyzed by SDS-polyacrylamide gel electrophoresis...
Mean Fura-PE3 and stimulated with indicated concentrations of ELC-Flag. K562 cells stably transfected with EBI1 were loaded with Fura-PE3 and stimulated with indicated concentrations of ELC-Flag. Mean ± S.E. from three separate experiments are plotted as percent maximal responses. The calculated EC50 is 1 nM.

and silver staining, the purified protein migrated as a single band of approximately 12 kDa (Fig. 4B). Analysis of the NH2-terminal amino acid sequence demonstrated that the mature ELC-Flag started at Gly-22 of the predicted sequence, as expected (not shown).

Specific Binding of ELC to EBI1—We first examined the binding of ELC to the five human CC chemokine receptors (CCR1 to 5) and five orphan receptors, V28/CMBKLR1 (47, 48), GPR-CY4a, GPR-9–6b, EBI1 (40), and BLR1 (49). To prepare labeled ELC convenient for binding assay, we generated an expression vector encoding the ELC fused with SEAP tagged with (His)6 (38). Alkaline phosphatase activity was useful for quantitative tracing, and the (His)6 tag in the COOH-terminus was used for affinity purification by nickel-agarose. ELC-SEAP was secreted by 293/EBNA-1 transfected with the expression vector as a protein with an apparent molecular mass of 73 kDa (not shown). Analysis of the NH2-terminal amino acid sequence of ELC-SEAP purified by nickel-agarose affinity chromatography revealed that the secreted ELC-SEAP started properly at Gly-22. K562 cells stably expressing CCR1 to 5 and four orphan receptors (BLR1 not included) were reacted with ELC-SEAP. As shown in Fig. 5, ELC-SEAP was found to bind specifically to EBI1 (40). No such binding was seen with K562 cells transfected with the vector only or those transfected with CCR1 to 5 or other three orphan receptors. Similar results were obtained by using 293/EBNA-1 cells stably transfected with the same set of cloned receptors including BLR1 (data not shown). As shown in Fig. 6, by displacement experiments, ELC-Flag fully competed with ELC-SEAP for EBI1 with an IC50 of 18 nM. In contrast, no other CC chemokines such as MCP-1 (66, 67), MCP-2 (63), MCP-3 (64), eotaxin (30), MIP-1α/LD78α (62), MIP-1β (60), RANTES (61), TARC (53), and LARC (38) were capable of competing with ECL-Flag for EBI1 (Fig. 6B). These results indicated that ELC is a highly specific high affinity ligand for EBI1.

**Fig. 7. Calcium mobilization of EBI1-transfected cells by ELC-Flag.** A, tracing profile of intracellular calcium flux. K562 cells stably transfected with EBI1 were loaded with Fura-PE3 and stimulated with 100 nM of ELC-Flag as indicated by arrowheads. A representative experiment from three separate experiments is shown. B, dose-response curve of calcium transients in EBI1-transfected cells stimulated by ELC-Flag. K562 cells stably transfected with EBI1 were loaded with Fura-PE3 and stimulated with indicated concentrations of ELC-Flag. Mean ± S.E. from three separate experiments are shown. Analysis of the NH2-terminal amino acid sequence demonstrated that the mature ELC-Flag started at Gly-22 of the predicted sequence, as expected (not shown).

**Fig. 8. Chemotactic response of EBI1-expressing cells to ELC-Flag.** A, chemotactic response of 293/EBNA-1 cells stably transfected with the vector alone (closed square) or the plasmid expressing EBI1 (closed circle). B, chemotactic response of HUT78 to ELC-Flag. Cells are stimulated with indicated concentrations of ELC-Flag by using a 48-well chemotaxis chamber. The assay was done in triplicate, and the number of migrating cells in five high power fields (×400) was counted for each well. Representative results of three separate experiments are shown.

**Induction of Calcium Mobilization in EBI1-Transfected Cells**—We next examined whether ECL-Flag was capable of inducing calcium mobilization in cells expressing EBI1. As shown in Fig. 7A, ELC-Flag induced calcium flux in K562 cells stably expressing EBI1 with complete desensitization for a rapid successive stimulation with ELC-Flag. ELC-Flag did not induce any calcium flux in parental K562 cells or those transfected with the vector alone (data not shown). The dose-response curve revealed an EC50 of 0.9 nM.

**Induction of Chemotaxis in EBI1-Expressing Cells**—We next examined the chemotactic responses of cells expressing EBI1 to ELC-Flag. As shown in Fig. 8A, 293/EBNA-1 cells stably transfected with EBI1 but not with the vector alone responded to ELC-Flag with a typical bimodal dose-response curve with a maximal effect at 300 ng/ml. We also tested a human T cell line HUT78 that expressed endogenous EBI1 at high levels (data not shown). The chemotactic response of 293/EBNA-1 cells stably transfected with EBI1 but also HUT78 cells expressing endogenous EBI1 responded to ELC-Flag by cell migration.

**Chromosomal Mapping of the ELC Gene**—The chromosomal location of the ELC gene was investigated by PCR using a DNA panel of somatic cell hybrids, each containing a single human chromosome. Unlike other CC chemokines, the ELC gene was localized on chromosome 9 (Fig. 9). To map the ELC gene on chromosome 9 more precisely, the radiation hybrid mapping was carried out. The results showed that the gene was located 164 centi-Ray away from the top of the chromosome and between the chromosomal markers D9S1978(WI-8765) and AFM326VD1 that are mapped at 9p13 (68) (Fig. 9B).

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5 This can be found at Chromosome 9 Home Page at http://www.gene.ucl.ac.uk/chr9home.html.
K562 cells with an EC50 of 0.9 nM (Fig. 7). ELC-Flag induced not by other CC chemokines so far tested (Fig. 6). ELC-Flag cells was competed only by ELC-Flag with an IC50 of 18 nM and shown). The binding of ELC-SEAP to EBI1-transfected K562 (Fig. 3). ELC-SEAP bound specifically to K562 cells stably

oid tissues such as thymus, lymph nodes, appendix, and spleen activity (Fig. 2). ELC is constitutively expressed in various lymph-
ticities from other standard CC chemokines. TARC, which

Recently, however, we have identified two novel human CC
genes are also clustered on chromosome 17q11.2 (1, 2, 45).

However, the roles of ELC and EBI1 in EBV-infected B cells and HHV6- or HHV7-infected T cells are not known at present but

ances of selectivity and specificity and may have biological activities on infected cells such as growth

We are grateful for Drs. Yorio Hinuma, Masakazu Hatanaka, and Retsu Miura for constant support and encouragement.

FIG. 9. Chromosomal localization of the human ELC gene. A, PCR analysis of a DNA panel of somatic cell hybrids, each containing a single unique human chromosome. The DNA panel of NIGMS human ×

rodent somatic cell hybrids was analyzed for the presence of the ELC gene by PCR. Lanes are labeled 1–22, X, and Y to indicate the human chromosome retained in each hybrid. DNA controls from human (H), Chinese hamster (C) and mouse (M), negative control without DNA (N), and positions of size markers (S) are indicated. Note the strong signal present in lanes 9 and H just above the nonspecific signal present in all lanes. B, radiation hybrid mapping. The area localized by radiation hybrid mapping is shown by a thick line along the ideogram of chromosome 9. The GeneBridge 4 panel consisting of DNA samples from 93 hybrids was used. The PCR results were: 0100001011 0001010110 1001010000 1001000000 0010110011 1001100010 1100100100 0001010000 0001010000 010 (Whitehead Institute/MIT Center for Genomic Research order). 0 and 1 represent negative and positive in the PCR assay, respectively.

DISCUSSION

The EST data bases (44) are useful sources for identification of new members of gene families including chemokines (38, 45). In the present study, we have described a novel human CC chemokine termed ELC from EB11-ligand chemokine. ELC shows homologies to other CC chemokines with 20–30% iden-
tity (Fig. 2). ELC is constitutively expressed in various lymph-
oid tissues such as thymus, lymph nodes, appendix, and spleen (Fig. 3). ELC-SEAP bound specifically to K562 cells stably transfected with EBI1 (Fig. 5). This was also confirmed by using 293/EBNA-1 cells stably transfected with EBI1 (not shown). The binding of ELC-SEAP to EBI1-transfected K562 cells was competed only by ELC-Flag with an IC50 of 18 nM and not by other CC chemokines so far tested (Fig. 6). ELC-Flag induced transient calcium mobilization in EBI1-transfected K562 cells with an EC50 of 0.9 nM (Fig. 7). ELC-Flag induced chemotactic responses in 293/EBNA-1 cells stably transfected with EBI1 and HUT78 cells expressing endogenous EBI1 at high levels (41) with a typical bimodal dose-response curve with a maximal effect at 300 ng/ml (Fig. 8). Collectively, ELC is a specific high affinity biological ligand for EBI1 (40). Since EBI1 was also shown to be constitutively expressed in various lymphoid tissues and on activated T and B lymphocytes (40, 41), ELC and EBI1 may play roles not only in inflammatory and immunological responses but also in normal lymphocyte recirculation and homing. It remains to be seen what types of cells produce ELC in various lymphoid tissues and what kinds of cytokines regulate ELC production. We propose EBI1 to be a specific high affinity biological ligand for EBI1 now enables us to examine the possible roles of ELC and EBI1 in infection and life cycle of herpesviruses. Such studies may lead to a new strategy against herpesvirus infection.

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Molecular Cloning of a Novel Human CC Chemokine EBI1-ligand Chemokine That Is a Specific Functional Ligand for EBI1, CCR7

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