Identification of CCR6, the Specific Receptor for a Novel Lymphocyte-directed CC Chemokine LARC*

Masatake Baba‡, Toshio Imai‡, Miyuki Nishimura‡, Mayumi Kakizaki‡, Shin Takagi‡, Kunio Hieshima§, Hisayuki Nomiyama§, and Osamu Yoshie‡

From the ‡Shionogi Institute for Medical Science, 2-5-1 Mishima, Setsusu-shi, Osaka 566, Japan and the Departments of §Biochemistry and Internal Medicine, Kumamoto University Medical School, Honjo, Kumamoto 860, Japan

Liver and activation-regulated chemokine (LARC) is a recently identified CC chemokine that is expressed mainly in the liver. LARC functions as a selective chemotactant for lymphocytes that express a class of receptors specifically binding to LARC with high affinity. To identify the receptor for LARC, we examined LARC-induced calcium mobilization in cells stably expressing five CC chemokine receptors (CCR1-CCR5) and five orphan seven-transmembrane receptors. LARC specifically induced calcium flux in K562 cells as well as 293/EBNA-1 cells stably expressing an orphan receptor GPR-CY4. LARC induced migration in 293/EBNA-1 cells stably expressing GPR-CY4 with a bi-modal dose-response curve. LARC fused with secreted alkaline phosphatase (LARC-SEAP) bound specifically to Raji cells stably expressing GPR-CY4 with a Kd of 0.9 nM. Only LARC but not five other CC chemokines (MCP-1, RANTES, MIP-1α, MIP-1β, and TARC) competed with LARC-SEAP for binding to GPR-CY4. By Northern blot analysis, GPR-CY4 mRNA was expressed mainly in spleen, lymph nodes, appendix, and fetal liver among various human tissues. Among various leukocyte subsets, GPR-CY4 mRNA was detected in lymphocytes (CD4+ and CD8+ T cells and B cells) but not in natural killer cells, monocytes, or granulocytes. Expression of GPR-CY4 mRNA in CD4+ and CD8+ T cells was strongly up-regulated by IL-2. Taken together, GPR-CY4 is the specific receptor for LARC expressed selectively on lymphocytes, and LARC is a unique functional ligand for GPR-CY4. We propose GPR-CY4 to be designated as CCR6.

The chemokines are a group of structurally related approximatively 70–90-amino acid polypeptides involved in leukocyte recruitment and activation (1, 2). The chemokines are grouped into two main subfamilies, CXC and CC, on the basis of the arrangement of the N-terminal two conserved cysteine residues. One amino acid separates the two cysteines in the CXC chemokines while the two cysteines are adjacent in the CC chemokines. Most CXC chemokines are potent neutrophil attractants while most CC chemokines recruit monocytes and also lymphocytes, basophils, and/or eosinophils with variable selectivity. Recently, a novel lymphocyte-specific chemotactic cytokine, lymphotactin/SCM-1, has been reported, which carries only the second and the fourth of the four cysteine residues conserved in all other chemokines (3, 4). This may suggest the existence of the C type chemokine subfamily.

The specific effects of chemokines on leukocytes are known to be mediated by a family of seven-transmembrane G-protein-coupled receptors (5). In humans, five CXC chemokine receptors and five CC chemokine receptors have been cloned and defined for their ligand specificity. They are CXCR1 for IL-8 (7); CXCR2 for IL-8 and other CC chemokines with the ELR motif (8–10); CXCR3 for IP-10 and MIG (11); CXCR4 for SDF-1/PBSF (12, 13); CCR1 for MIP-1α, RANTES, and MCP-3 (14–17); CCR2 for MCP-1, MCP-3, and MCP-4 (17–19, 44); CCR3 for Eotaxin, RANTES, MCP-3, and MCP-4 (20–23, 44); CCR4 for TARC (24, 25); CCR5 for RANTES, MIP-1α, and MIP-1β (26, 27, 45). Furthermore, there are a growing number of putative chemokine receptors whose ligands remain to be identified.

Recently, we have identified a novel CC chemokine, LARC (liver and activation-regulated chemokine), and mapped its gene to chromosome 2q33-q37 (28). Expression of LARC mRNA was detected mainly in the liver among various human tissues and also induced in several human cell lines by phorbol 12-myristate 13-acetate. LARC was chemotactic for lymphocytes but not for monocytes. LARC fused with the secreted alkaline phosphatase (LARC-SEAP) bound specifically to lymphocytes with a Kd of 0.4 nM. Notably, the binding of LARC-SEAP was competed only by LARC and not by other chemokines so far tested (28). These results indicated the presence of a class of receptors specific for LARC on lymphocytes. In the present study, we have demonstrated that an orphan receptor GPR-CY4 is the LARC receptor that is selectively expressed on lymphocytes.

EXPERIMENTAL PROCEDURES

Cells—Human hematopoietic cell lines were maintained in RPMI 1640 supplemented with 10% fetal calf serum (FCS). 293/EBNA-1 cells were purchased from Invitrogen (San Diego, CA) and maintained in Dulbecco's modified Eagle's medium supplemented with 10% FCS. Peripheral blood leukocytes were fractionated by surface markers as de-motif 1; G-protein, heterotrimeric guanine nucleotide-binding regulatory protein; CXCR, CXC chemokine receptor; IL-8, interleukin 8; IP-10, interferon-γ-inducible protein 10; MIG, monokine induced by interferon-γ; SDF-1, stroma derived factor 1; PBSF, pre-B cell stimulatory factor; CCR, CC chemokine receptor; MCP, macrophage inflammatory protein; RANTES, regulated on activation, normal T cell expressed and secreted; MCP; monocyte chemotactant protein; TARC, thymus and activation-regulated chemokine; LARC, liver and activation-regulated chemokine; SEAP, secreted alkaline phosphatase; FCS, fetal calf serum; PBMC, peripheral blood mononuclear cells; NK, natural killer; BSA, bovine serum albumin; EB1, EBV-induced gene 1; BLR1, Burkitt's lymphoma receptor 1; IL-2, interleukin 2; PHA, phytohemagglutinin; HBSS, Hank's balanced salt solution; PBL, peripheral blood leukocytes.  
1 From the Shionogi Institute for Medical Science, 2-5-1 Mishima, Setsusu-shi, Osaka 566, Japan.

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* To whom correspondence should be addressed: Shionogi Institute for Medical Science, 2-5-1 Mishima, Setsusu-shi, Osaka 566, Japan. Tel.: 81-6-382-2612; Fax: 81-6-382-2598; E-mail: osamu.yoshie@shionogi.co.jp.

† The abbreviations and trivial names used are: SCM-1, single C

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suspended in HBSS-BSA at 2.5 × 10^6 cells/ml. 2 ml of the cell suspen-
dium with appropriate forward and side scatters were sorted on a
FACStar Plus (Beckton Dickinson, Mountain View, CA) as a
natural killer (NK) cells. CD4+ T cells and CD8+ T cells were purified
from PBMC by negative selection using Dynabeads (Dynal Oslo, Nor-
way) or by incubation with anti-CD16, -CD14, -CD20, and -CD8, or
-CD16, -CD14, -CD20, and -CD4, respectively. Granulocytes were ob-
tained from the pellet fraction of Ficoll-Paque gradient by dextran
sedimentation and hypotonic lysis of erythrocytes. The purity of each
cell population was always >95% as determined by flow cytometry or by
staining with Diff-Quik (Baxter Scientific Products, McGav Park, IL).

Chemokines—Recombinant LARC, TARC, Eotaxin, and MCP-1 were
produced by using a baculovirus expression system and purified as
described previously (20, 28, 29). MIP-1α and MIP-1β were purchased
from Pepro Tech (Rocky Hill, NJ). LARC fused with the secreted form
of alkaline phosphate tagged with six histidine residues, LARC-SEA-
P(His)6, was prepared and purified as described previously (28). In
brief, the LARC cDNA was subcloned into the SEAP(His)6 vector
(pDREF-SEAP(His)6-Hygro28), making the expression vector pDREF-
LARC-SEAP. 293/EBNA-1 cells were transfected with pDREF-LARC-
SEAP using LipofectAMINE (Life Technologies, Inc., Gaithersburg,
MD) and cultured for 3–4 days in DMEM containing 10% FCS. The
stable LARC-SEAP clones were selected by x-radiation and were
maintained with 1% BSA and placed in lower wells (25 × 10^3 cells/ml).

Stable Expression of Cloned Receptors—Cells stably expressing
CCR1 (14–17), CCR2B (17–19), CCR3 (20–23), CCR4 (24, 25), CCR5
(26, 27, 45), V28/CMKBR1 (30, 31), GPR-CY4 (GenBankTM accession
number U45984), GPR-9-6 (GenBankTM accession number:U45982),
EBI1 (32), and BLR1 (33) were described previously (25). In brief, the
expression plasmids based on pDREF-Hyg (29) were transfected into
Raji cells by electroporation and into 293/EBNA-1 cells by Lipo-
fectAMINE (Life Technologies, Inc.) using LipofectAMINE (Life
Technologies, Inc., Gaithersburg, MD) and cultured for 3–4 days in
DMEM containing 10% FCS. The culture supernatants were centrifuged,
filtered (0.45 μm), added to 20 mM HEPES, pH 7.4, and 0.02% sodium
azide, and stored at 4 °C.

Calcium Mobilization Assay—This was carried out as described pre-
viously (25). In brief, cells were suspended in 3 × 10^5 cells/ml in Hank’s
balanced salt solution (HBSS) containing 1 mg/ml of bovine serum
albumin (BSA) and 10 mM HEPES, pH 7.4, (HBSS-BSA) and incubated
with 1 μM fura-PE3-AM (Texas Fluorescence Labs) at room tempera-
ture for 1 h. Cells were washed twice with HBSS-B2, cells were sus-
pered in HBSS-BSA at 2.5 × 10^5 cells/ml. 2 ml of the cell suspension
in a quartz cuvette was placed in a luminescence spectrometer
(Perkin-Elmer LS 50B) and fluorescence was monitored at 340 nm
(λex1), 380 nm (λex2) and 510 nm (λem) every 200 ms. To determine EC50,
dose-response curve was generated in each experiment by plotting
data as percent maximum response.

Migration Assay—Cell migration was determined by using a 48-well
microchemotaxis chamber as described previously (29). In brief, each
chemo-attractant was diluted in Hepes-buffered RPMI 1640 supple-
mented with 1% BSA and placed in lower wells (25 μl/well). Cells
suspended in RPMI 1640 with 1% BSA at 2 × 10^5 cells/ml were placed
in upper wells (50 μl/well). Upper and lower wells were separated by a
polyvinylpyrrolidone-free polycarbonate filter with 8-μm pores pre-
coated with type IV collagen. Incubation was carried out at 37 °C for 4 h
in 5% CO2, 95% air. Filters were removed, washed, and stained with
Diff-Quik. Migrated cells were counted in five randomly selected high-
power fields (400 ×) per well. All determinations were done in
triplicate.

Binding Assay—This was carried out as described previously (25, 28,
29). In brief, for displacement experiments, 2 × 10^5 cells were incubated
for 1 h at 16 °C with 1 nM of SEAP(His)6 or LARC-SEAP(His)6 in the
presence of 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. For saturation experiments, cells were incubated for 1 h at
16 °C with increasing concentrations of LARC-SEAP(His)6 in the
presence or absence of 1 μM unlabeled LARC. After incubation, cells
were washed five times and lysed in 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 phosphatase. After brief centrifugation to remove
cell debris, alkaline phosphatase activity in 10 μl of lysate was mea-
sured by chemiluminescent assay as described previously (28). All
determinations were done in duplicate. The binding data were analyzed
by the LIGAND program (34).

RESULTS

Induction of Calcium Mobilization by LARC—To examine
interaction of LARC with each cloned receptor, we measured
LARC-induced calcium mobilization in a panel of K562 cells stably
expressing the five known CCRs (CCR1-CCR5) and five
orphan chemokine receptors, V28/CMKBR1 (31, 32), EBI1
(33), BLR1 (34), GPR-CY4 (GenBankTM accession number

FIG. 1. Calcium mobilization in cells expressing GPR-CY4 by
LARC. A, K562 cells stably transfected with indicated cloned receptors
were loaded with fura-PE3-AM and stimulated with indicated chemo-
kines at 100 nM (LARC and TARC) or 10 nM (MIP-1α, MCP-1, eotaxin,
and MIP-1β). Arrowheads indicate time of application of chemokines.
Intracellular calcium concentration was monitored by fluorescence ratio
(F340/F380). Representative results from at least three separate
experiments are shown. B, dose-response curve of calcium mobilization
by LARC. 293/EBNA-1 cells stably transfected with GPR-CY4 were
loaded with fura-PE3-AM and stimulated with indicated concentrations
of LARC. Results are expressed as percent maximum responses. Each
point represents mean ± S.E. from three separate experiments.
U45984), and GPR-9–6 (GenBank™ accession number U45982). As shown in Fig. 1A, LARC specifically induced calcium flux in K562 cells expressing GPR-CY4 with complete desensitization against a rapid successive treatment with LARC. LARC did not induce calcium flux in parental K562 cells or those expressing CCR1, CCR2B, CCR3, CCR4, CCR5, or four other orphan receptors. On the other hand, MIP-1α, MIP-1β, MCP-1, eotaxin, or TARC did not induce calcium flux in K562 cells expressing GPR-CY4 (not shown). These chemokines, however, properly induced calcium flux in K562 cells expressing their respective CCRs even after treatment with LARC (Fig. 1A). Similar results were obtained by using a panel of 293/EBNA-1 cells stably expressing these cloned receptors (data not shown). As shown in Fig. 1B, 293/EBNA-1 cells stably expressing GPR-CY4 with complete desensitization against a rapid successive treatment with U45982). As shown in Fig. 1B, 293/EBNA-1 cells stably expressing GPR-CY4 responded to LARC in calcium mobilization with an EC50 of ~50 nM. These results clearly demonstrated that LARC was a specific functional ligand for GPR-CY4.

**Induction of Chemotaxis by LARC**—Previously, we showed that LARC induced chemotaxis in freshly isolated peripheral blood lymphocytes with a maximal effect at 1 μg/ml (28). We therefore examined whether LARC was capable of inducing migration of 293/EBNA-1 cells stably expressing GPR-CY4. As shown in Fig. 2A, LARC induced migration in cells stably expressing GPR-CY4 with a typical bi-modal dose-response curve with a maximum effect at 1 μg/ml and an EC50 of ~100 ng/ml (~12 nM). LARC did not induce migration in cells transfected with the vector alone. A checkerboard-type analysis revealed that the migration of GPR-CY4-transfected 293/EBNA-1 cells toward LARC was mostly chemotactic (Fig. 2B).

**Binding of LARC**—Previously, we showed that LARC-SEAP(His)6 specifically bound to a single class of receptors expressed on lymphocytes with a Kd of 0.4 nM (28). Importantly, the binding of LARC-SEAP(His)6 was competed only by LARC and not by any other chemokines so far tested, indicating that the LARC receptor is not shared by other chemokines (28). We therefore examined the binding of LARC-SEAP(His)6 to a panel of Raji cells stably expressing GPR-CY4 and other cloned receptors. LARC-SEAP(His)6 bound specifically to cells expressing GPR-CY4 but not to parental cells or those expressing five CCRs or four other orphan receptors (data not shown). As shown in Fig. 3A, the binding of LARC-SEAP(His)6 to GPR-CY4 was saturable when increasing concentrations of LARC-SEAP(His)6 were incubated with Raji cells expressing GPR-CY4. The Scatchard analysis revealed a Kd of 0.9 nM and 28,800 sites/cell (Fig. 3B). Unlabeled LARC fully competed with

![FIG. 2. Chemotactic response of GPR-CY4-transfected cells to LARC. A, 293/EBNA-1 cells stably transfected with GPR-CY4 (closed circle) or the vector alone (open triangle) were tested for in vitro migration to indicated concentrations of LARC 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×) were counted for each well. Representative results from three separate experiments are shown. Each point represents mean ± S.E. B, a checkerboard-type analysis of cell migration. In the chemotaxis assay using a 48-well chemotaxis chamber, LARC was added to top and/or bottom wells as indicated at 100 ng/ml. The assay was done in triplicate, and the number of migrating cells in five high power fields (400×) were counted for each well. Representative results from three separate experiments are shown. Each histogram represents mean ± S.E.](http://www.jbc.org/)

![FIG. 3. Binding characteristics of LARC-SEAP(His)6 to GPR-CY4-transfected cells. A, specific binding of LARC-SEAP(His)6 to GPR-CY4. Raji cells stably transfected with GPR-CY4 (2 × 10⁶ cells) were incubated at 16 °C for 1 h with increasing concentrations of LARC-SEAP(His)6. Specific binding was determined by subtracting nonspecific binding measured in the presence of 1 nM of LARC. Representative results from three separate experiments are shown. Each point represents mean ± S.E. B, Scatchard analysis of the binding data in panel A. The calculated Kd is 0.9 nM. C, displacement of binding of LARC-SEAP(His)6 to GPR-CY4 by LARC. Raji cells stably expressing GPR-CY4 (2 × 10⁶ cells) were incubated with 1 nM of LARC-SEAP(His)6 in the presence of increasing concentrations of unlabeled LARC. Representative results from three separate experiments are shown. The calculated IC50 is 3.4 nM. D, competition of LARC-SEAP(His)6 binding to GPR-CY4 by various CC chemokines. Raji cells stably transfected with GPR-CY4 (2 × 10⁶ cells) were incubated with 1 nM LARC-SEAP(His)6 in the presence of indicated chemokines at 200 nM. Each histogram represents mean ± S.E. from three separate experiments.](http://www.jbc.org/)
4). When blots for various tissues were hybridized with the tissues and leukocyte subsets by Northern blot analysis (Fig. 4), the expression pattern of GPR-CY4 in various human tissues was found to be expressed strongly in the spleen and weakly in the lymph nodes. Weak expression was also detected in the appendix, and weakly in the fetal liver (Fig. 4). When the expression of GPR-CY4 mRNA in various human tissues. Multi-tissue Northern blot filters (2 μg of poly(A)+ RNA/ lane) (CLONTECH) were hybridized with the 32P-labeled GPR-CY4 cDNA probe. B, expression of GPR-CY4 and LARC in various human lymphoid tissues. Immune blot filters (2 μg/ lane of poly(A)+ RNA) (CLONTECH) were hybridized with the 32P-labeled GPR-CY4 probe. The same filters were rehybridized with the 32P-labeled LARC cDNA probe.

LARC-SEAP(His)6 for GPR-CY4 with an IC50 of 3.4 nM (Fig. 3D). These binding characteristics were highly consistent with those obtained from the endogenous class of LARC receptors expressed on lymphocytes (28).

Selective Expression of GPR-CY4 in T and B Cells—We have shown that the endogenous class of LARC receptors is expressed selectively on lymphocytes (28). Therefore, we examined the expression pattern of GPR-CY4 in various human tissues and leukocyte subsets by Northern blot analysis (Fig. 4). When blots for various tissues were hybridized with the 32P-labeled GPR-CY4 cDNA probe (Fig. 4A), GPR-CY4 mRNA was found to be expressed strongly in the spleen and weakly in the lymph nodes. Weak expression was also detected in the testis (larger transcripts), small intestine, and PBL. Notably, the mRNA expression was very low, if any, in the liver where the LARC transcripts were mainly detected (28). When blots specific for various lymphoid tissues were probed, GPR-CY4 mRNA was detected strongly in the spleen, lymph nodes, and appendix, and weakly in the fetal liver (Fig. 4B). When the same lymphoid tissue blots were rehybridized with the 32P-labeled LARC cDNA probe, LARC mRNA was detected moderately in the appendix and weakly in the lymph nodes, PBL, and fetal liver (Fig. 4B). Thus, the constitutive expression of GPR-CY4 and that of LARC overlap partially in the secondary lymphoid tissues. We then examined the expression of GPR-CY4 mRNA in various leukocyte subsets. T cells (both CD4+ and CD8+ T cells) and B cells were clearly positive, whereas NK cells, monocytes, or granulocytes were virtually negative even though some RNA loading differences were noted (Fig. 5A). We also examined the expression of GPR-CY4 mRNA in various human hematopoietic cell lines. Only a T cell line, Hut102, weakly expressed GPR-CY4, whereas other T cell lines (Molt-4, Jurkat, and Hut78), monocytic cell lines (THP-1 and U937), B cell lines (Raji and Daudi), an erythroleukemia cell line (K562), a promyelocytic cell line (HL-60), a basophilic cell line (KU812), and a megakaryocytic cell line (MEG-1) were virtually negative (not shown). Collectively, the expression of GPR-CY4 is mostly limited in the secondary lymphoid tissues and also in T and B lymphocytes. The expression pattern of GPR-CY4 is thus highly consistent to the lymphocyte-selective expression of the endogenous LARC receptor described previously (28).

Loetscher et al. (35) have reported that CD45RO+ T cells express CCR1 and CCR2 only after prolonged treatment with IL-2. They further showed that activation of T cells with PHA, anti-CD3, or anti-CD3 and anti-CD28 did not induce expression of CCR1 or CCR2 but rather suppressed the effect of IL-2 (35). We therefore examined the effect of IL-2 without or with PHA on expression of GPR-CY4 in CD4+ and CD8+ T cells (Fig. 5B). Expression of GPR-CY4 in both CD4+ and CD8+ T cells was strongly up-regulated by IL-2. The effect of IL-2 was, however, strongly suppressed by co-treatment with PHA. Thus, the expression of GPR-CY4 in T cells is positively regulated by IL-2 but negatively regulated by T-cell activation like those of CCR1 and CCR2 (35).

DISCUSSION

LARC is a novel CC chemokine with 20–28% identity to other cloned human CC chemokines (28). LARC is mainly expressed in the liver and also induced in human cell lines, such as a monocytic cell line U937, by phorbol myristate acetate. Thus, we designated this chemokine as LARC from Liver and Activation-Regulated Chemokine (28). The present study has further demonstrated that LARC is constitutively expressed at relatively low levels in tissues such as the lymph
nodes, appendix, and fetal liver (Fig. 4B). It remains to be explored what types of cells produce LARC in the liver and some lymphoid tissues and what kinds of cytokines and stimulants regulate LARC expression.

LARC is chemotactic for lymphocytes in vitro with a maximal activity at 1 μg/ml (28). At high concentrations, LARC may also be chemotactic for neutrophils. However, LARC is totally inactive on monocytes (28). A similar relatively low potency in induction of chemotaxis in lymphocytes has been noted for TARC (29) and SDF-1/PBSF (36). Lymphocytes, especially resting ones, may be relatively inefficient in chemotactic responses to these chemokines. In keeping with the lymphocyte-selective activity of LARC, lymphocytes possess a class of receptors binding LARC with a high affinity ($K_d = 0.4$ nM) (28). Furthermore, the receptor expressed on lymphocytes is highly specific for LARC and not shared by other CC chemokines so far tested (28). Interestingly, TARC (29) and SDF-1/PBSF (36) are also the ones that possess receptors, CCR4 (25) and CXCR4 (12, 13), respectively, that are not shared by other chemokines so far tested.

In the present study, we have demonstrated that an orphan receptor GPR-CY4 (GenBank™ accession number U45984) is the LARC receptor expressed on lymphocytes. Recently, the same receptor was also deposited in the data base as DRY6 (GenBank™ accession number U60000). It was also reported as an orphan receptor CKR-L3 (37). LARC induced calcium mobilization and chemotactic responses specifically in K562 cells and 293/EBNA-1 cells stably expressing GPR-CY4 (Figs. 1 and 2). LARC fused with SEAP bound specifically to Raji cells stably expressing GPR-CY4 with a $K_d$ of 0.9 nM (Fig. 3). Blocking of LARC-SEAP to GPR-CY4 was blocked only by LARC and not by any other chemokines so far tested (Fig. 3). GPR-CY4 was found to be expressed strongly in the secondary lymphoid tissues such as the spleen, lymph nodes, and appendix, and also in the fetal liver (Fig. 4). A very similar result was reported for the tissue expression of CCR-L3 (37). Furthermore, GPR-CY4 was expressed highly selectively in peripheral blood lymphocytes, namely both CD4+ and CD8+ T cells and B cells (Fig. 5A). Collectively, these results clearly indicate that GPR-CY4 is the receptor that specifically binds LARC with high affinity and is expressed selectively on lymphocytes. We propose GPR-CY4 to be designated as CCR6.

Compared with the high affinity binding of LARC to CCR6 ($K_d = 0.9$ nM), LARC needed much higher concentrations to induce intracellular calcium mobilization ($EC_{50} = 50$ nM) or chemotactic responses ($EC_{50} = 12$ nM) in cells stably transfected with CCR6. At present, we do not know the exact causes of such discrepancies, but these may be due in part to differences in assay conditions such as temperature, duration of incubation, etc. Furthermore, Monteciaro and Charo (46) recently demonstrated a two-step mechanism for activation of CCR1 by MCP-1 in which high affinity binding of MCP-1 with the amino terminus of CCR1 allows subsequent low affinity interactions of MCP-1 with the extracellular loops/transmembrane domains of CCR1 that lead to receptor activation and signaling. A similar two-step mechanism may explain high affinity binding versus low signaling potency of LARC to CCR6.

As in the cases of CCR1 and CCR2 (35), IL-2 strongly induces the expression of CCR6 in resting T cells whereas PHA activation blocked the inducing effect of IL-2 (Fig. 5B). Thus, not antigenic stimulation per se, but subsequent IL-2-mediated expansion may enhance T-cell responsiveness to LARC. In mice, repeated injection of IL-2 was shown to induce massive lymphocyte infiltration in the liver and lung (38). Since LARC is expressed rather selectively in the liver and lung (38), LARC may be involved in the IL-2-induced lymphocyte infiltration in these organs.

Most CC chemokines are known to interact with multiple shared receptors (1, 2, 5, 6). For example, MIP-1α binds to CCR1 and CCR5 (14, 15, 26, 27, 45), while RANTES binds to CCR1, CCR3, and CCR5 (14, 15, 21, 22, 26, 27, 45). Eotaxin apparently interacts only with CCR3 (20–22), but CCR3 also binds RANTES, MCP-3, and MCP-4 (21–23, 44). Thus, each chemokine may recruit multiple types of cells even if they express different types of receptors, whereas each cell may respond to multiple types of chemokines even by expressing a single type of receptor. The exact physiological meanings of such redundant and complex relationships between chemokines and their receptors are still unclear, but such partially overlapping specificities may have advantages in acute inflammatory responses where similar leukocyte subsets have to be rapidly recruited in a wide variety of settings and microenvironments even if there are considerable differences in the local pattern and spectrum of chemokine production. In this context, LARC (28) and also the recently identified T-cell-directed CC chemokine TARC (29) are quite unique because they interact with highly specific receptors, CCR6 (this paper) and CCR4 (25), respectively. In fact, LARC and TARC have a number of features in common that are unique among the known CC chemokines. They are constitutively expressed in certain organs and lymphoid tissues, with LARC mainly in the liver (28) and also in some secondary lymphoid tissues (Fig. 4B), whereas TARC is mainly expressed in the thymus and also most probably in some secondary lymphoid tissues (29). Both LARC and TARC act selectively on lymphocytes, LARC on both T and B cells (28, Fig. 5), whereas TARC acts mainly on CD4+ T cells (25, 29). Even though the genes for other CC chemokines are known to be clustered on human chromosome 17q11.2 (1, 2), the genes for LARC and TARC are mapped distinctly to chromosomes 2q33-q37 and chromosome 16q13, respectively (28, 39). Thus, LARC and TARC may constitute a new group of CC chemokines that have more specialized functions in lymphocyte trafficking and immune responses than other CC chemokines clustered on chromosome 17.

In this regard, the CXC chemokine SDF-1/PBSF is also the one that acts via its specific receptor CXCR4 (12, 13), is constitutively expressed in various tissues (40), and is mapped to chromosomes 10q (40) instead of chromosome 4 where the genes for other CXC chemokines are known to be clustered (1, 2). Furthermore, the recently described C type chemokine lymphotactin/SCM-1 (3, 4) that also acts selectively on lymphocytes is distinctly mapped to human chromosome 1q23 (41). By generating gene-targeted mice, SDF-1/PBSF has been shown to be essential for B cell lymphopoiesis in the fetal liver and for myelopoiesis and B cell lymphopoiesis in the bone marrow during embryonic development (42). Thus, during embryogenesis, SDF-1/PBSF may be involved in generation of B cell progenitors in the fetal liver and in colonization of hematopoietic precursor cells into the bone marrow (42). Recently, gene-targeted mice lacking a putative CXC chemokine receptor BLR1 that is expressed on mature B cells and a subpopulation of CD4+ T cells have been shown to have anatomical defects such as lack of inguinal lymph nodes, impaired development of Peyer’s patches, and defective formation of primary follicles and germinal centers in the spleen (43). When injected into wild type mice, B cells lacking BLR1 failed to migrate from the T cell-rich zone into B cell follicles in the spleen and Peyer’s patches (43). Thus, BLR1 may be involved in B cell migration within specific anatomic compartments in the spleen and Peyer’s patches. Likewise, TARC and LARC with their respective
receptors, CCR4 and CCR6, may play roles not only in inflammatory and immunological responses but also in the normal lymphocyte trafficking and microenvironmental homing that are essential for development and maintenance of various lymphoid tissues.

Identification of the LARC receptor CCR6 now enables us to define the exact types of cells that respond to LARC. Generation of gene-targeted mice lacking LARC and CCR6 will be useful to address their in vivo functions.

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Identification of CCR6, the Specific Receptor for a Novel Lymphocyte-directed CC Chemokine LARC
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