The T Cell-directed CC Chemokine TARC Is a Highly Specific Biological Ligand for CC Chemokine Receptor 4*

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Thymus and activation-regulated chemokine (TARC) is a recently identified CC chemokine that is expressed constitutively in thymus and transiently in stimulated peripheral blood mononuclear cells. TARC functions as a selective chemoattractant for T cells that express a class of receptors binding TARC with high affinity and specificity. To identify the receptor for TARC, we produced TARC as a fusion protein with secreted alkaline phosphatase (SEAP) and used it for specific binding. By stably transfecting five orphan receptors and five known CC chemokine receptors (CCR1 to -5) into K562 cells, we found that TARC-SEAP bound selectively to cells expressing CCR4. TARC-SEAP also bound to K562 cells stably expressing CCR4 with a high affinity (Kd = 0.5 nM). Only TARC and not five other CC chemokines (MCP-1 (monocyte chemoattractant protein-1), RANTES (regulated upon activation, normal T cells expressed and secreted), MIP-1α (macrophage inflammatory protein-1α), MIP-1β, and LARC (liver and activation-regulated chemokine)) competed with TARC-SEAP for binding to CCR4. TARC but not RANTES or MIP-1α induced migration and calcium mobilization in 293/EBNA-1 cells stably expressing CCR4. K562 cells stably expressing CCR4 also responded to TARC in a calcium mobilization assay. Northern blot analysis revealed that CCR4 mRNA was expressed strongly in human T cell lines and peripheral blood T cells but not in B cells, natural killer cells, monocytes, or granulocytes. Taken together, TARC is a specific functional ligand for CCR4, and CCR4 is the specific receptor for TARC selectively expressed on T cells.

Chemokines are small secreted polypeptides that play important roles in a wide range of inflammatory and immunological processes by recruiting selected subsets of leukocytes (1, 2). The known chemokines are divided into two major subfamilies based on the spacing of the first two cysteines in the conserved motif. The CXC chemokine subfamily, which includes IL-8 (3) and IP-10 (4), is characterized by the presence of a single amino acid separating the first two cysteines. The two cysteines are adjacent in the CC chemokine subfamily, which includes RANTES (5), MCP-1 (6, 7), MCP-2 (8), MCP-3 (9), MCP-4 (10), MIP-1α (11), MIP-1β (12), I-309 (13), eotaxin (14, 15), HCC-1 (16), TARC (17), and LARC (18). The CXC chemokines preferentially attract and activate neutrophils, whereas the CC chemokines usually attract and activate monocytes and also basophils, eosinophils, or lymphocytes with variable selectivity (19). Recently, lymphotactin/single C motif 1 that carries only the second and the fourth of the four cysteine residues conserved in other chemokines has been identified, suggesting the existence of the C type chemokine subfamily (20, 21). The human genes for the CXC, CC, and C chemokines are clustered on human chromosomes 4, 17, and 1, respectively (1, 22, 23). Recent studies indicate that genes for certain chemokines are present outside these clusters. For example, a CXC chemokine SDF-1/PBSF has been mapped to human chromosome 10 (24), and CC chemokines TARC and LARC have been mapped to human chromosomes 16 and 2, respectively (18, 25). In addition to chemotactic activity, some chemokines have a regulatory activity on hematopoiesis and angiogenesis (26–28). Recently, it has been shown that three CC chemokines, MIP-1α, MIP-1β, and RANTES, block infection of macrophage-tropic strains of human immunodeficiency virus type 1, while a CXC chemokine, SDF-1/PBSF, blocks infection of T cell line-tropic human immunodeficiency virus type 1 strains (29, 30).

The specific effects of chemokines on target cells are mediated by seven-transmembrane G-protein-coupled receptors (31). To date, at least five human CC chemokine receptors have been defined for ligand specificity. CCR1 is a receptor for MIP-1α, RANTES, and MCP-3 (32–35); CCR2 is a receptor for MIP-1α and MCP-3 (35, 36); CCR3 is a receptor for eotaxin, RANTES, and MCP-3 (14, 37, 38); CCR4 is a receptor for MIP-1α, RANTES, and MCP-1 (39); and CCR5 is a receptor for MIP-1α, MIP-1β, and RANTES (40–42). The specific ligands for CCR1, CCR2, CCR3, and CCR5 were demonstrated by specific binding and functional assays such as chemotaxis and calcium flux using cDNA-transfected mammalian cells. In the case of CCR4, however, only marginal levels of binding of MIP-1α and RANTES were shown with HL-60 cells transfected with CCR4 (43), while a chloride current induction in response to MIP-1α, RANTES, and MCP-1 was demonstrated in CCR4 cRNA-injected oocytes (39). Except for CCR3 that is almost exclusively expressed on eosinophils (38, 44), other receptors were reported to be expressed on monocytes and lymphocytes. Notably, CCR4 that was originally cloned from a human basophilic cell line was shown to be expressed selectively in thymus besides peripheral blood mononuclear cells (PBMCs) (39).
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Recently, we have isolated a novel CC chemokine, TARC (thymus and activation-regulated chemokine) by an efficient signal sequence trap using an Epstein-Barr virus vector (17). TARC is expressed transiently in phytohemagglutinin-stimulated PBMC and constitutively and selectively in thymus. TARC induces chemotaxis in certain human T cell lines but not in monocytes or granulocytes. Pretreatment of cells with Pertussis toxin abolishes cell migration induced by TARC. Radio-labeled recombinant TARC specifically binds to T cell lines and peripheral blood T cells but not to monocytes or granulocytes. The binding of radiolabeled TARC to T cells is competed by TARC but not by other chemokines such as IL-8, MIP-1α, RANTES, or MCP-1. These results indicate the existence of a class of highly specific Pertussis toxin-sensitive G-protein-coupled receptors for TARC on T cells. In the present study, we have demonstrated that CCR4 is the specific high affinity functional receptor for TARC that is expressed selectively on T cells.

EXPERIMENTAL PROCEDURES

Cells—Human hematopoietic cell lines were maintained in RPMI 1640 supplemented with 10% fetal calf serum (FCS). The murine pre-B cell line L1.2 was kindly provided by Dr. Craig Gerard (Harvard Medical School, Boston, MA) and maintained in RPMI 1640 supplemented with 10% 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. PBMCs were isolated from venous blood obtained from healthy adult donors using Ficoll-Paque (Pharmacia, Uppsala, Sweden). Monocytes were stained with fluorescein isothiocyanate-conjugated anti-CD14 and positively selected by MACS (Miltenyi Biotec, Bergisch, Germany). B cells were stained with fluorescein isothiocyanate-conjugated anti-CD19 and positively selected by MACS. T cells were stained with fluorescein isothiocyanate-conjugated anti-CD3 and positively selected by MACS. Natural killer (NK) cells were sorted by FACStar Plus (Beckton Dickinson, Mountain View, CA) as CD16+ or CD56+ and CD3- cells with appropriate forward and side scatters. Purification of CD4+ and CD8+ T cells from PBMCs was carried out by negative selection using Dynabeads (Dynal, Oslo, Norway) after incubation of PBMCs with anti-CD16, anti-CD14, anti-CD20, and anti-CD8 or anti-CD4, respectively. Granulocytes were obtained from the pellet fraction of Ficoll-Paque gradient by dextran sedimentation and hypotonic lysis of erythrocytes. The purity of lymphocytes, T cells, and NK cells was monitored at 340 nm (95–99% as determined by flow cytometric analysis or by staining with Diff-Quik (Baxter Scientific Products, McGaw Park, IL).

Construction of Receptor Expression Plasmids—The cDNA fragments covering the open reading frames for various chemokine receptors and orphan receptors were obtained as follows. Cloning of CCR3 cDNA was described previously (14). EB1 and BLR1 cDNA were isolated by screening a phytohemagglutinin-stimulated human PBMC cDNA library. Other receptors were cloned from a human PBMC cDNA library or human genomic library by polymerase chain reaction (PCR). The primers were designed using the sequences from the following GenBank™ submissions: CCR1 (L07520), CCR2B (U09389), CCR4 (X65740), CCR5 (X91492), CXCR4 (M99299), V2X (U03505), GPR-CY4 (U45984), GPR-9-6 (U45982), EB11 (L35181), BL1R (X58149). The fragments were cloned into an expression vector pDRF-Hygro (17) for efficient expression in 293/EBNA-1 cells or Raji cells, or pCAG-Neo (kindly provided by T. Nakajima) for expression in K562 cells and L1.2 cells.

Stable Transfection—For stable expression in Raji cells, cells were transfected by electroporation as described previously (17). For stable expression in 293/EBNA-1 cells, 1 × 106 cells were plated on 100-mm dishes. After 12–20 h, cells were transfected with recombinant plasmids (10 μg each) using LipofectAMINE (Life Technologies, Inc.). After selection with 250 μg/ml hygromycin for 1–2 weeks, drug-resistant cells were pooled and used for experiments. For stable expression in K562 cells and L1.2 cells, 1 × 106 cells in 500 μl of phosphate-buffered saline were electroporated with 10 μg of linearized plasmid at 260 V, 960 μF (Biorad, Richmond, CA) and 0.5% sodium azide. For one-step affinity purification of TARC, cells were eluted with 100 mM imidazol. The concentration of TARC-SEAP(His)6 was determined by a sandwich type enzyme-linked immunosorbent assay as described previously (18). Briefly, 96-well microtiter plates (Nunc Maxisorb) were coated with antiphosphatase monoclonal antibody (Medix Biotech, Foster City, CA). After blocking with 1 mg/ml bovine serum albumin (BSA) in phosphate-buffered saline, the samples were titrated and incubated for 1 h at room temperature. After washing, plates were incubated with biotinylated rabbit antiphosphatase alkaline phosphatase diluted 1:500 for 1 h at room temperature, washed again, and incubated with peroxidase-conjugated streptavidin (Vector) for 30 min. After washing, bound peroxidase was reacted with 3,3′,5,5′-tetramethylbenzidine. Reaction was stopped by adding 1 M HOAc, and absorbance at 450 nm was measured. Alkaline phosphatase activity was determined by a chemiluminescent assay using the Great Esc ape detection kit (CLONTECH). Purified antiphosphatase alkaline phosphatase (Cosmo Bio) was used to generate a standard curve. The enzymatic activity was expressed as relative light units/s. The SEAP(His)6 and TARC-SEAP(His)6 used in the present study had a specific activity of approximately 8.88 × 103 relative light units/s and 1.25 × 104 relative light units/s per pmol, respectively.

Binding Assay—For displacement experiments, cells were incubated for 1 h at 16°C with 1 nM of SEAP(His)6 or TARC-SEAP(His)6 in the presence of increasing concentrations of unlabeled chemokines in 200 μl of RPMI 1640 containing 20 μM Hepes (pH 7.4). 1% BSA and 0.02% sodium azide. For saturation experiments, cells were incubated at 16°C with increasing concentrations of TARC-SEAP(His)6 in the presence or absence of 1 μM unlabeled TARC. After incubation, cells were washed five times and lysed in 50 μl of 10 mM Tris-HCl (pH 8.0), 1% Triton X-100. Samples were heated at 65°C for 10 min to inactivate cellular phosphatases. The purified protein A-Sepharose CL-4B (Pharmacia) was added to the alkaline phosphatase activity in 25 μl of lysate was determined by the chemiluminescent assay described above. For direct binding experiments, cells were incubated for 1 h at 16°C with 0.1 nM of 125I-RANTES, 125I-MCP-1, 125I-MIP-1α, or TARC-SEAP(His)6 without or with 200 nM of unlabeled chemokines in 200 μl of low salt binding buffer (50 mM Hepes, pH 7.5, 1 mM CaCl2, 5 mM MgCl2, 0.5% BSA, and 0.05% sodium azide). The cells were washed three times, washed for 1 h on ice, washed twice, washed for 1 h on ice, and then washed four times with low salt binding buffer containing 0.5 M NaCl. All assays were done in duplicate. Binding data were analyzed by the LIGAND program (45).

Migration Assay—Cell migration was assayed by using a 48-well microchemotaxis chamber as described previously (17, 34). In brief, chemotactants were diluted in Hepes-buffered RPMI 1640 supplemented with 1% BSA and placed in lower wells (25 μl/well). Cells were suspended in the same medium at 2 × 106 cells/ml and added to upper wells (50 μl/well). The upper and lower wells were separated by a polyvinylpyrrolidone-free polycarbonate filter with 8-μm pores that was precoated with type IV collagen (34). The chamber was incubated for 4 h at 37°C in 5% CO2, 95% air. Filters were removed and stained with Diff-Quik. Each sample was assayed in triplicate, and migrated cells were counted in five randomly selected high power fields (×400) per well.

Calcium Mobilization Assay—Cells were suspended at 3 × 105 cells/ml in Hank’s balanced salt solution supplement with 1 mg/ml BSA and 10 mM HEPES, pH 7.4. Cells were incubated with 1 μM fluo-3/AM (Texas Fluorescence Labs) at room temperature for 1 h in the dark. After washing twice, cells were resuspended at 2.5 × 106 cells/ml. To measure intracellular calcium, cells in 2 ml were placed in a quartz cuvette in a Perkin-Elmer LS 50B spectrofluorometer. Fluorescence was monitored at 340 nm (ex1), 380 nm (ex2), and 510 nm (em) every 200 ms. A dose-response curve was generated in each experiment, and results were expressed as percentage of maximum response.

Northern Blot Analysis—Total RNAs were prepared from various subsets of peripheral blood leukocytes using Trizol (Life Technologies, Inc.).
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results

Use of TARC-Alkaline Phosphatase Fusion Protein for Receptor Binding Assay—Chemokines often have a tendency for self-aggregation at physiological pH and toxicity that interferes with their receptor studies (46, 47). To circumvent this problem, Luster et al. generated IP-10 fused with SEAP and showed that the fusion protein retained its ability to interact specifically with cell surface receptors without self-aggregation in physiological buffers (46). To prepare labeled TARC convenient for receptor binding experiments, we decided to adopt this method and expressed TARC as a fusion protein with SEAP (P(His)6) for CCR4 (Fig. 4A). Since TARC was shown not to share the major class of its high affinity ligand for TARC receptors, we carried out TARC-SEAP(His)6 binding experiments using another human T cell line Hut78. The binding of TARC-SEAP(His)6 was fully competed by unlabeled TARC with an IC50 of 0.5 nM and 29,000 sites/cell was demonstrated (Fig. 4A). These results may indicate that Hut78 expresses at least two types of TARC receptors, one highly specific for TARC and another shared by RANTES and LARC. At any rate, the present results were highly consistent with those obtained previously by using 125I-labeled TARC. Thus, TARC-SEAP(His)6 was proven to retain its ability as a specific high affinity ligand for TARC receptors.

CCR4 Is a High Affinity Specific Receptor for TARC—Since TARC was shown to not share the major class of its high affinity binding sites on T cells with other CC chemokines that are known to bind to one or more CCRs (17), we first tested the binding of TARC-SEAP(His)6 to orphan receptors such as EBI1 (48), BLR1 (49), V28 (50), GPR9–6 (GenBank accession number: HSU45982), GPR-CY4 (GenBank accession number: HSU45984), and LESTR/fusin (51). LESTR/fusin is now known as the receptor for SDF-1/PBSF (CXCR4) (52, 53). TARC-SEAP(His)6 showed no significant binding to Raji, 293/EBNA-1, or K562 cells stably transfected with any of these orphan receptors (data not shown). In parallel experiments, we also examined the binding of TARC-SEAP(His)6 to the five CC chemokine receptors that are known to be shared by several CC chemokines (CCR1 to -5). Surprisingly, TARC-SEAP(His)6 specifically bound to CCR4 expressed on K562 (Fig. 3), Raji, and 293/EBNA-1 (not shown). To further characterize the binding of TARC to CCR4, experiments were performed using K562 cells stably expressing CCR4. When the binding was performed with increasing concentrations of TARC-SEAP(His)6 (Fig. 4A), a single class of receptors with a Kd of 0.5 nM and 29,000 sites/cell was demonstrated (Fig. 4B). Competition experiments showed that unlabeled TARC fully competed with TARC-SEAP(His)6 for CCR4 (Fig. 4C). In contrast, other CC chemokines such as RANTES, MCP-1, MIP-1α, MIP-1β, and LARC showed

2 We also confirmed that TARC-SEAP(His)6 induced calcium flux in CCR4-transfected K562 cells with similar potency as TARC.
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no significant competition with TARC-SEAP(His)_6 for CCR4 (Fig. 4D). Similar results were obtained using Raji or 293/EBNA-1 cells stably transfected with CCR4 (data not shown). Collectively, the binding characteristics of TARC-SEAP(His)_6 to CCR4 were highly consistent with those obtained with the major class of the endogenous TARC receptors expressed on T cells (Fig. 2) (17).

We next compared direct binding of TARC-SEAP(His)_6, RANTES, MCP-1, and MIP-1α to CCR4-transfected cells (Fig. 5). In these experiments, murine L1.2 cells transfected with CCR4 were used (38). As expected, TARC-SEAP(His)_6 specifically bound to CCR4-transfected L1.2 cells at levels about 20-fold higher than those obtained with Hut78 cells that express endogenous TARC receptors at 1000–2000 sites/cell (Fig. 2). In contrast, RANTES, MCP-1, or MIP-1α bound to CCR4-transfected L1.2 cells at marginal levels, if any, although these chemokines efficiently bound to THP-1 cells that express endogenous receptors at 1000–5000 sites/cell (1). These results further strengthen the possibility that TARC is the physiological ligand for CCR4.

TARC Induces Chemotaxis in CCR4-transduced Cells—Previously, we showed that TARC induced chemotaxis in certain human T cell lines (17). We therefore examined whether TARC also induced migration of cells expressing transfected CCR4. 293/EBNA-1 cells were stably transfected with CCR4 or CCR1, and migration of these cells to TARC, MIP-1α, and RANTES was examined. As shown in Fig. 6, TARC induced migration of cells transfected with CCR4 but not those transfected with CCR1. On the other hand, MIP-1α and RANTES induced migration of cells transfected with CCR1 but not those transfected with CCR4. Parental 293/EBNA-1 cells or those transfected with the vector alone did not respond to TARC, MIP-1α, or RANTES (data not shown). Migration of CCR4-transfected 293/EBNA-1 cells to TARC was concentration-dependent, being promoted at concentrations from 10 to 1000 ng/ml. Desensitization was observed at 10 μg/ml (not shown). A checkerboard analysis indicated that the migration of CCR4-transfected 293/EBNA-1 cells toward TARC was mostly chemotactic but partly (~40%) chemoattractant (data not shown). Collectively, TARC but not MIP-1α or RANTES is a functional ligand for CCR4.
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On the other hand, K562 cells expressing CCR1 responded to MIP-1α but not to TARC, whereas K562 cells expressing CCR2B responded to MCP-1 but not to TARC. Parental K562 cells or those transfected with the vector alone did not show any response to TARC, RANTES, MIP-1α, or MCP-1 (data not shown). Responses to TARC were detectable above 1 nM, and maximum values were obtained at 100 nM with an EC50 of 8 nM (Fig. 7B). We further confirmed that TARC at 100 nM did not induce calcium fluxes in CCR1, CCR2B, CCR3, or CCR5-transfected K562 cells, while MIP-1α induced calcium fluxes in CCR1- and CCR5-transfected cells, MCP-1 in CCR2B-transfected cells, and eotaxin in CCR3-transfected cells (data not shown). Similar results were obtained by using 293/EBNA-1 cells. These observations again demonstrated that TARC is a functional ligand for CCR4.

**DISCUSSION**

Although a number of chemokines are known to act on T cells (54–61), TARC appears to be the first CC chemokine highly...
selective for T cells (17). High levels of constitutive expression of TARC have been detected only in thymus and not in spleen. High levels of specific binding sites for TARC have been detected on some T cell lines and peripheral blood T cells but not on monocytes or granulocytes. TARC has been shown to induce chemotaxis in certain human T cell lines. Furthermore, a class of TARC receptors expressed on T cells is highly specific for TARC and not shared by any other CC or CXC chemokines that are known to act on T cells (17). Here we have presented several lines of evidence indicating that CCR4 is the major class of receptors for TARC that is selectively expressed on T cells and not shared by other CC or CXC chemokines.

CCR4 was originally cloned by Power et al. from a human basophilic cell line KU-812 (39). The CC chemokines, MIP-1α, RANTES, and MCP-1, were presumed to be the functional ligands for CCR4, because among various chemokines, only these were able to activate a calcium-dependent chloride channel in Xenopus laevis oocytes injected with CCR4 cRNA. However, induction of chemotaxis or calcium flux in CCR4-transfected mammalian cells by MIP-1α, RANTES, and MCP-1, or any other chemokines has not been demonstrated. Here we have shown that introduction of the CCR4 cDNA into Raji, 293/ EBNA-1, and K562 cells induced a class of high affinity binding sites for TARC (Figs. 3 and 4). Binding of TARC to CCR4 was competed only by TARC and not by any other chemokines including MIP-1α, RANTES, and MCP-1 (Fig. 4). Binding of 125I-labeled RANTES, MCP-1, or MIP-1α to CCR4 were marginal, if any, while they bound to endogenous receptors expressed on THP-1 cells efficiently (Fig. 5). Furthermore, only TARC but not RANTES, MCP-1, or MIP-1α induced chemotaxis in CCR4-transfected 293/EBNA-1 and induced calcium flux in CCR4-transfected 293/EBNA-1 and K562 (Figs. 6 and 7). Collectively, these results have clearly demonstrated that CCR4 is the specific functional ligand for CCR4. The discrepancy between Power et al. (39) and the present study is probably due to the assay system used in the former study, i.e. activation of a calcium-dependent chloride channel in Xenopus laevis oocytes injected with CCR4 cRNA. Besides CCR4, however, some T cells may also express a class of receptors for TARC that is shared by RANTES and LARC (Fig. 2).

Compared with the high affinity value obtained from binding of TARC-SEAP/His$_6$ to CCR4 ($K_d = 0.5$ nM), the potency of TARC in induction of chemotaxis in CCR4-transfected 293/EBNA-1 (EC$_{50} = 10$ nM) or in calcium flux in CCR4-transfected K562 cells (EC$_{50}$, $8$ nM) appeared to be considerably less. Previously, we observed that TARC induced chemotactic responses in two human T cell lines Hut78 and Hut102 with an EC$_{50}$ of about 2 nM (17). Types and/or efficiency of G-proteins coupling to CCR4 may be different depending on the cell background. SDF-1/PBSF is another chemokine that was reported to require high concentrations in induction of chemotaxis in lymphocytes and monocytes (53). Recently, Montecarlo and Charo proposed a two-step mechanism for activation of the MCP-1 receptor CCR2 in which high affinity binding of MCP-1 with the receptor amino terminus allows subsequent low affinity interactions with the extracellular loops/transmembrane domains that lead to receptor activation and signaling (62). A similar two-step mechanism may apply to high affinity binding versus relatively low potency in activation of CCR4 by TARC. Furthermore, like I-309 that was shown to be much more potent in inhibition of glucocorticoid-induced apoptosis of murine T cell lymphomas than in induction of chemotaxis in human THP-1 monocytic cells (63), TARC may be more potent in some biologic activities other than induction of chemotaxis or calcium mobilization.

Although several chemokine receptors are known to be expressed on T cells, CCR4 appears to be the first CC chemokine receptor highly selective for T cells. By Northern blot analysis, CCR4 mRNA was detected highly selectively in some T cell lines and peripheral blood T cells, especially CD4$^+$ T cells, but not in B cells, NK cells, monocytes, or granulocytes (Fig. 8). Previously, Power et al. (39) also demonstrated by Northern blot analysis that CCR4 was expressed strongly in thymus and peripheral blood leukocytes but very weakly in spleen. However, they further demonstrated by reverse transcriptase-PCR analysis that CCR4 was expressed not only in T cells but also in B cells and monocytes. Taken together, T cells are the cells that express CCR4 at high levels, but other types of leukocytes may also express CCR4 at low levels detectable by reverse transcriptase-PCR. The selective expression of CCR4 on T cells is thus consistent with the fact that T cells are the major target of TARC. Furthermore, the fact that TARC and CCR4 are both constitutively and strongly expressed in thymus further supports their important roles in trafficking and education of thymocytes within the thymus.

Notably, we also detected expression of CCR4 in a basophilic cell line KU812 and a megakaryocytic cell line MEG-1 (Fig. 8). In fact, CCR4 was originally cloned from KU812, and, by using reverse transcriptase-PCR, Power et al. (39) demonstrated expression of CCR4 in fresh basophils, especially after brief treatment with IL-5. They also mentioned that platelets contain high levels of CCR4 mRNA (39, 64). We have also detected high levels of TARC-binding sites on platelets (data not shown). Thus, it is clear that cells of the megakaryocyte/platelet lineage also express CCR4. It remains to be seen whether TARC affects differentiation and/or function of basophils and megakaryocytes/platelets besides T cells.

It is also noteworthy that high levels of CCR4 expression are observed only in certain human T cell lines and peripheral blood T cells of especially the CD4 type. CCR4 may thus be expressed selectively in particular subsets of T cells and/or in particular stages of differentiation and/or activation of T cells. Cultured CD45RO$^+$ T cells were shown to express CCR1 and CCR2 in a strictly IL-2-dependent manner (65). Both CD4$^+$ and CD8$^+$ T cells were shown to constitutively express CCR5 (41). Activated T cells were shown to express CXCR3, the first T cell-selective CXC chemokine receptor shared by IP-10 and Mig (66). It is thus important to determine phenotypes of T cells that express CCR4 and conditions that regulate CCR4 expression. Obviously, elucidation of the physiological roles of the TARC/CCR4 system will be greatly facilitated by generation of mutant mice lacking the respective genes.

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