Molecular Cloning of a Novel T Cell-directed CC Chemokine Expressed in Thymus by Signal Sequence Trap Using Epstein-Barr Virus Vector*

Toshibo Imai, Tetsuya Yoshida, Masataka Baba, Miyuki Nishimura, Mayumi Kakizaki, and Osamu Yoshiie

From the Shionogi Institute for Medical Science, 2-5-1 Mishima, Settsu-shi, Osaka 566, Japan.

Precursors of most secreted and cell surface molecules carry signal sequences at their amino termini. Here we describe an efficient signal sequence trap method and isolation of a novel CC chemokine. An expression library was constructed by inserting 5' portion-enriched cDNAs from phytohemagglutinin-stimulated peripheral blood mononuclear cells into upstream of signal sequence-deleted CD4 cDNA in an EB virus shuttle vector. After electroporation into Raji cells, CD4 antigen-positive cells were enriched by repeated cell sorting and plasmids were recovered in Escherichia coli. Out of 100 plasmid clones examined, 42 clones directed expression of CD4 antigen on the cell surface. Among them were signal sequences of CD6, β2-microglobulin, MGC-24, and T cell receptor ϵ-chain, and at least four novel potential signal sequences. A cDNA clone encoding a novel CC chemokine was isolated by using one of the trapped fragments. The gene designated as TARC from Thymus and Activation-Regulated Chemokine was expressed transiently in phytohemagglutinin-stimulated peripheral blood mononuclear cells and constitutively in thymus. Radiolabeled recombinant TARC specifically bound to T cell lines and peripheral T cells but not to monocytes or granulocytes. The binding of radiolabeled TARC to the high-affinity receptor (Kd, 2.1 nm) on Jurkat was displaced by TARC but not by interleukin-8, MIP-1α, RANTES, or MCP-1. TARC also bound to the promiscuous chemokine receptor on erythrocytes (Kd, 17 nm). TARC induced chemotaxis in T cell lines Hut78 and Hut102. Pretreatment of Hut78 with pertussis toxin abolished the TARC-induced cell migration. Collectively, T cells express a highly selective receptor for TARC that is coupled to pertussis toxin-sensitive G-protein. TARC may factor playing important roles in T cell development in thymus as well as in trafficking and activation of mature T cells.

Emigration of leukocytes from blood into sites of inflammation and immune responses is essential for host defense mechanisms. Local tissue irritation causes leukocytes to stick to blood vessels, to pass through them, and finally to accumulate at irritated sites. It is now known that a family of cytokines called chemokines play important roles in recruiting selected subsets of leukocytes and are involved in a wide range of acute and chronic inflammatory processes as well as other immunoregulatory and hematopoietic functions (1, 2). The known chemokines are divided into two major subfamilies based on the spacing of the first two cysteines in the conserved motif and the chromosomal localization of their genes. The α or XCR 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, and the human genes are clustered on chromosome 4q12–21. The two cysteines are adjacent in the β or CC chemokine subfamily, which includes RANTES (5), MCP-1 (6, 7), MCP-2 (8), MCP-3 (9), MIP-1α (10), MIP-1β (11), I-309 (12), and eotaxin (13), and the human genes are located on chromosome 17q11–12. Neutrophils are preferentially attracted and activated by members of the CC chemokine subfamily, whereas monocytes are preferentially attracted and activated by members of the CC chemokine subfamily. A number of recent studies have revealed that certain β chemokines attract basophils, eosinophils, and lymphocytes with variable selectivity (14–18). Recently, two novel chemokine-like molecules, mouse lymphotactin and human SCM-1, have been described (19, 20). These cytokines carry only the second and fourth of the four cysteines conserved in other chemokines, suggesting the existence of the γ or C type chemokine subfamily. The chemotactic activity of lymphotactin/SCM-1, however, remains to be rigorously proven.

The specific effects of chemokines on the target cell are mediated by seven-transmembrane G-protein-coupled receptors (21). To date, at least five human CC chemokine receptors have been identified by cDNA cloning. CC CKR1 is a receptor for MIP-1α, RANTES, and MCP-3 (22, 23); CC CKR2A for MCP-1 (24); CC CKR2B for MCP-1 and MCP-3 (24); CC CKR3 for eotaxin (13); CC CKR4 for MIP-1α, RANTES and MCP-1 (25); CC CKR5 for MIP-1α, MIP-1β, and RANTES (26). In addition to these receptors, a promiscuous chemokine receptor which binds both CXC and CC chemokines is found mainly on the surface of erythrocytes (27–29).

Recently, a new cloning method aiming at selective identifi-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank/EMBL Data Bank with accession number(s) D43767.

† To whom correspondence and reprint requests should be addressed: Shionogi Institute for Medical Science, 2-5-1 Mishima, Settsu-shi, Osaka 566, Japan. Tel.: 81-6-382-2612; Fax: 81-6-382-2598; E-mail: toshio.imai@shionogi.co.jp.
cDNA species encoding secretory proteins and type I membrane proteins was introduced (30). The method coined as signal sequence trap took advantage of the presence of NH2-terminal signal sequences in most precursor forms of secretory proteins and type I transmembrane proteins, which are necessary for the proper orientation of the NH2-terminal of mature forms inside endoplasmic reticulum and exocytotic vesicles. This method enables to selectively clone cDNA species encoding intercellular signal-transducing molecules without biologic assays. In the present study, we have developed an efficient signal sequence trap method based on an Epstein-Barr virus shuttle vector and applied it to phathemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC). Novel cDNA fragments encoding potential signal sequences were identified and one of them led to identification of a novel CC chemokine that is constitutively expressed in thymus and highly selective for T cells.

EXPERIMENTAL PROCEDURES

Cell lines—Human hematopoietic cell lines were maintained in RPMI 1640 supplemented with 10% fetal calf serum. 293/EBNA-1 (Invitrogen, San Diego, CA) and HeLa cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. PBMC were isolated from venous blood obtained from healthy adult donors using Ficoll-Paque (Pharmacia, Uppsala, Sweden). Monocytes were purified by positive selection using anti-CD14 antibody and MiniMACS (Miltenyi Biotec, Bergisch, Germany). Lymphocytes were obtained by centrifugation on an iso-osmotic Percoll gradient as described (31). Human peripheral blood T lymphocytes were purified by negative selection using anti-CD16, anti-CD14, and anti-CD20 antibody with Dynal beads (Dynal, Oslo, Norway), and were stimulated with PHA (1:100) (Life Technologies, Inc., Gaithersburg, MD) and PMA (100 ng/ml) (Sigma). Granulocytes were purified from the pellet of Ficoll-Paque gradient by dextran sedimentation and lysis of erythrocytes. Erythrocytes were isolated from the cells by alkaline lysis procedure and recovered into Escherichia coli. The plasmids prepared from individual colonies were reintroduced into Raji and clones that were able to directed expression of CD4 antigen on the cell surface were identified. The length of insert fragments were determined by PCR using EF seq F primer (5’-CCATCGTCAATCAGACCAGA-3’) and CD4ST seq R primer (5’-TGATCGTCGAGGCTCACTGTG-3’). The cDNA fragments longer than 200 bp were analyzed by sequencing.

Isolation of Full-length cDNA Clone—A conventional cDNA library of PBMC stimulated with PHA for 72 h was constructed using cDNA synthesis kit (Life Technologies, Inc.). The library was screened with DNA of the trapped fragments containing secretory sequences, and one positive clone D3A was isolated. The full-length clone and subclones were sequenced using Autorated Sequence kit and A.L.F. sequencer (Pharmacia).

Northern Blotting—Poly(A)+ RNA was prepared from PBMC were separated by electrophoresis on a 1% agarose gel containing 0.66 M formaldehyde. The gel was blotted to a filter membrane (Hybond N+) (Amersham, Arlington Heights, IL) and hybridized with 32P-labeled full-length cDNA probes purchased from Clontech. Hybridization was carried out at 42°C with 50% formamide, 5×SSPE, 2% SDS, and 100 µg/ml sonicated salmon sperm DNA. The probe was the Smal-PstI fragment of clone D3A. Probes for other chemokines were prepared by reverse transcriptase-PCR and described previously (34). The β-actin probe was purchased from Clontech.

Expression of TARC in E. coli and Generation of Anti-TARC Antibodies—The fragment encoding the predicted mature form of TARC was prepared by PCR from human PBMC RNA using primers (5’-GGCCGGGGGGCTCAAAG-3’, 5’-GGCGGGGGGACCAGACAGTGGTTCAAAG-3’) and cloned into the vector pET3d (Novagen, Madison, WI) at a SalI and NotI sites of pET3d. The recombinant E. coli BL21 cells transformed with pET-TARC construct were grown in Luria-Bertani medium (Difco) supplemented with 100 µg/ml ampicillin. After bacteria reached an OD600 of 0.8, the expression was induced by addition of 0.5 mM IPTG and the bacteria were harvested after 4 h. The bacterial pellets were sonicated in PBS containing 1% Triton X-100 and after centrifugation the supernatant was collected. The crude recombinant TARC was purified by Ni-Sepharose affinity chromatography and further purified by HiPrep 26/10 SizeExclusion column (Pharmacia).

Purification of TARC Expressed in a Bacterial System—S9 cells and TN5B1–4 cells (Invitrogen) were maintained at 27°C in EX-CELL 400 medium (JRH Biosciences, Lenexa, KS). The baculovirus transfer vector pVL1393 was purchased from Invitrogen. The TARC transfer vector, pVL-TARC, was made by ligating the EcoRI-NotI fragment of the clone D3A into EcoRI-NotI sites of pVL1393. A linearized AdMNPV DNA containing a lethal deletion (Clontech) and pVL-TARC were cotransfected into S9 cells and the recombinant baculovirus was isolated by limiting dilution. For the expression of recombinant TARC, TN5B1–4 cells were plated at 1.2 × 105 cells onto 150-mm flask and infected after 2 h at multiplicity of infection of 10–20 with the recombinant baculovirus. After 48 h of infection, the cells were harvested and the supernatants were collected and subjected to gel filtration (Sephacryl S-200) and adsorbed to Pansorbin followed by elution with PBS containing 0.5 M NaCl (Pharmacia). The purified TARC was concentrated to 1 mg/ml and the concentration was determined by BCA assay (Pierce).

Isolation of T1 in Human PBMC—Peripheral blood mononuclear cells (PBMC) from healthy adult donors were isolated and stimulated with PHA (1:100) (Life Technologies, Inc.) at a concentration of 106 cells/ml for 72 h. The supernatants were collected and TARC protein was measured by ELISA.

Expression of TARC in Human PBMC—Human PBMC were isolated and stimulated with PHA (1:100) (Life Technologies, Inc.) at a concentration of 106 cells/ml for 72 h. The supernatant was collected and TARC protein was measured by ELISA.
A Novel T Cell-specific CC Chemokine

Development of an Epstein-Barr Virus Vector-based Signal Sequence Trap Method—We generated a new signal sequence trapping vector, pDREF-CD4ST, which was based on an Epstein-Barr virus shuttle vector and contained the signal sequence-deleted CD4 cDNA for a reporter protein (Fig. 1A). Since the vector can be maintained as episomes in the presence of EBNA-1, this system allows an efficient stable expression of recombinant TARC in the presence or absence of a kanamycin-resistance gene for selection (hygR), the EBNA-1 gene, and the EBV origin for episomal replication (oriP). 5′-Terminal-ended cDNAs are inserted between SalI and XbaI sites and expressed as fusion proteins with signal sequence-deleted CD4. B, flow cytometric analysis of surface expression of CD30 signal sequence-CD4 fusion protein on Raji cells. Raji cells were transfected with pDREF-CD4ST containing the CD30 signal sequence and cultured in the presence of 200 μg/ml hygromycin for 3 days. The cells were stained by indirect immunofluorescence method using anti-CD4 (closed profile). The background fluorescence was obtained by staining only with fluorescence isothiocyanate-labeled anti-mouse IgG (open profile).

Cloning of cDNA Fragments Encoding Signal Sequences from PHA-stimulated Human PBMC—We generated 5′ portion-enriched cDNA fragments from PHA-stimulated human PBMC by using the 5′-rapid amplification of cDNA ends method (33) coupled with random-primed first-strand cDNA synthesis. The cDNA fragments of around 300 to 600 bp were inserted into pDREF-CD4ST, and about 10^6 independent clones were obtained. The expression library was then transfected into Raji cells and stable transformants were selected. The initial frequency of the CD4 antigen-positive cells was approximately 0.1% as determined by flow cytometric analysis. After expansion, CD4 antigen-negative cells were sorted and enriched to 18.2%. The antigen-positive cells increased to 29.7% after the second expansion and sorting, and to 44.8% after the third expansion and sorting. No appreciable increase in the CD4 antigen-positive cells was obtained by the fourth expansion and sorting. We therefore rescued plasmids from the cells into E. coli. Plasmids were prepared from randomly selected 100 colonies and individually reintroduced into Raji cells. We identified 42 clones that were capable of directing surface expression of CD4 antigen. Considering that short fragments might fortuitously encode hydrophobic sequences in unnatural open reading frames, we only sequenced 36 clones.
MGC24 (42), TCR 9 oligo(dC)-positive clones. These were derived from CD6 (41), mitochondrialDNA. Signalsequences were present in 6 out of clones were negative for the oligo(dC) anchor and derived from thathad been introduced at 5
12 known clones, 9 clones were positive for the anchor oligo(dC) reported previously and 24 clones were unknown. Among the sequences with the data bases revealed that 12 clones were possessing inserts longer than 200 bp. Comparison of the sequences with the data bases revealed that 12 clones were reported previously and 24 clones were unknown. Among the 12 known clones, 9 clones were positive for the anchor oligo(dC) that had been introduced at 5’ ends of mRNA. The remaining 3 clones were negative for the oligo(dC) anchor and derived from mitochondrial DNA. Signal sequences were present in 6 out of 9 oligo(dC)-positive clones. These were derived from CD6 (41), MGC24 (42), TCR (43), and β2-microglobulin (3 independent clones) (44). Among 24 unknown clones, 13 clones were positive for the anchor oligo(dC). Hydrophathy analysis of the 13 oligo(dC)-positive clones revealed that at least 4 clones possessed hydrophobic profiles resembling to signal sequence.

Identification of a Novel CC Chemokine—We selected clone 98 for further study because it presented a characteristic feature of CC chemokine, i.e. a double-cysteine motif nine residues downstream of the putative signal sequence cleavage site (1, 2). In order to isolate the full-length cDNA, we screened a conventional cDNA library of PHA-stimulated PBMC with clone 98 and obtained a clone D3A (Fig. 2). The cDNA is 538 bp in length and has an identical sequence with the clone 98 in its 5’-overlapping region. The clone 98 had extra 12 nucleotides in the 5’ side. The sequence of clone D3A contains a single long open reading frame that starts with the 5’-proximal methionine codon at nucleotide 59 and encodes a highly basic polypeptide of total 94 amino acids with a calculated molecular weight of 10,507. The deduced polypeptide sequence contains a highly hydrophobic amino-terminal region characteristic of a signal peptide with a putative cleavage site between Ala-23 and Ala-24. The predicted mature protein has an isoelectric point of 9.7 and a molecular weight of 8,083. There are no potential N-glycosylation sites. The 3’-untranslated region contains a potential polyadenylation signal (ATTAA). The ATTAA motif which is frequently found in the 3’-noncoding sequences of cytokines and involved in rapid degradation of mRNA (45) is not observed. The predicted mature protein shows significant homology to CC chemokines and all the four cysteine residues conserved in the CC chemokine family are present (Fig. 2). The identity is 29% with RANTES (5), 28% with MIP-1β (11) and MCP-3 (9), 26% with MIP-1α (10), and 24% with I-309 (12), MCP-1 (6, 7) and MCP-2 (8). The protein encoded by clone D3A is thus a novel member of the CC chemokine subfamily.

Expression of the D3A mRNA in PHA-stimulated PBMC and Thymus—We examined expression of D3A mRNA in PBMC by Northern blot hybridization (Fig. 3A). The expression of D3A was undetectable in fresh PBMC. After stimulation with PHA, the transcripts of about 0.8 kilobases accumulated to maximum levels at 24 h and returned to low levels by 72 h. On the other hand, the transcripts for MIP-1α were present in fresh PBMC, rapidly increased by PHA-stimulation with a peak at 4 h, and returned to resting levels by 24 h. Next, we examined the expression of D3A mRNA in various tissues (Fig. 3B). The transcripts were detected strongly in thymus, and weakly in lung, colon, and small intestine. Compared to other five chemokines examined in parallel, D3A is quite unique for its constitutive expression in thymus and lack of such expression in spleen or PBL. From the constitutive expression in thymus and stringently regulated induction in PBMC by activation, we designated this novel member of the CC chemokine family as TARC (Thymus and Activation-Regulated Chemokine).

Secretion of TARC from HeLa Cells Transfected with the cDNA—To demonstrate TARC as a secretory protein, the TARC cDNA was expressed in HeLa using the vaccinia/T7 system. The culture supernatants from transfectedants were analyzed by immunoblotting using polyclonal antiserum prepared against glutathione S-transferase-TARC fusion protein produced in E. coli. A single protein of approximately 8 kDa was detected by the antiserum in the supernatant from HeLa transfected with the TARC cDNA but not in the supernatant from HeLa transfected with the vector alone (Fig. 4). The size of the secreted protein was close to the predicted value of the peptide with a putative cleavage site between Ala-23 and Ala-24.
mature TARC. Amino acid sequence analysis demonstrated that the NH₂ terminus of recombinant TARC secreted from HeLa started at Ala-24 of the predicted sequence. These results indicate that the predicted signal sequence and the cleavage site of TARC are functional and correct, respectively.

Purification of Recombinant TARC—Recombinant TARC was produced in Tn5B-1 insect cells infected with a recombinant baculovirus and purified from culture supernatants by cation-exchange chromatography and reverse-phase HPLC. Recombinant TARC was eluted from the reverse-phase column as a single peak (Fig. 5A). The yield of purified protein was typically 1–1.5 mg/ml of starting culture supernatant. When analyzed by SDS-PAGE and silver staining, the purified protein migrated as a single band of 8 kDa (Fig. 5B). Amino acid sequence analysis demonstrated that the NH₂ terminus of recombinant TARC started at Ala-24 of the predicted sequence, indicating that insect cells correctly removed the signal peptide (Fig. 5C). Immunoblot analysis showed that TARC derived from HeLa or insect cells comigrated with bacterial recombinant TARC lacking the signal sequence (Fig. 4). These results indicate that there are no post-translational modifications such as glycosylation or proteolytic processing in mature TARC.

Detection of TARC Receptors—As a first step for elucidation of the biological activity of TARC, we determined the distribution of receptors for TARC on various cell types (Fig. 6). A considerable specific binding of TARC was detected on a number of T cell lines, PBL, and peripheral blood T cells activated by PHA/PMA. On the other hand, myelomonocytic cell lines and peripheral monocytes showed only a marginal, if any, specific binding of TARC, while Raji, 293/EBNA-1, and peripheral granulocytes showed virtually no specific binding. The TARC receptor on T cells, further binding experiments were performed with Jurkat. Binding of radiolabeled TARC reached equilibrium by 1 h at 15°C. When the binding was performed with increasing concentrations of 125I-TARC (Fig. 7A), a single class of receptor with a Kₐ of 2.1 nM and 603 sites/cell was observed (Fig. 7B). Competition binding experiments showed that unlabeled TARC fully competed the binding of 125I-TARC (Fig. 7C). Scatchard analysis of the competition data showed a single class of receptor with a Kₐ of 2.1 nM and 948 sites/cell. None of the tested CXC and CC chemokines (IL-8, RANTES, MCP-1, and MIP-1α) showed significant competition for 125I-TARC (Fig. 7D). Similar results were obtained with Hut 78 cells and peripheral lymphocytes activated by PHA/PMA (data not shown).

It is known that erythrocytes possess a promiscuous chemo-
erythrocytes with similar dose-response profiles. The $K_d$ for TARC was 17 nM which is comparable to the reported values of other chemokines (28, 29). Collectively, these results indicate that TARC binds to a highly specific receptor(s) expressed mainly, if not exclusively, on T cells and to the promiscuous chemokine receptor expressed on erythrocytes.

Chemotactic Activity of TARC—Human T cell lines, Hut78, Hut102, monocytes, and neutrophils were analyzed for their migration in response to indicated concentrations of TARC (closed circles), medium only (closed squares), MCP-1 (only for monocytes) (closed triangle), and IL-8 (only for neutrophils) (closed circle), using a 48-well chemotaxis chamber as described under “Experimental Procedures.” The assay was done in triplicate and the number of migrating cells in five high-power fields (800 ×) was counted for each well. Each point represents mean ± S.D. Representative results from at least three separate experiments are shown.

A checkerboard analysis confirmed that the migration of Hut78 cells toward TARC was chemotactic rather than chemokinetically. Anti-TARC antibody raised by immunizing guinea pigs with recombinant TARC almost completely neutralized TARC in induction of chemotaxis in Hut78 (Fig. 10A). Finally, pretreatment of Hut78 with pertussis toxin abolished chemotactic response to TARC in a dose-dependent manner with IC$_{50}$ at 2 nM (Fig. 10B), indicating that the chemotactic response to TARC was mediated by a G$_o$- or G$_i$- subclass G-protein-coupled receptor. We, however, have not detected
TARC Ab

presence or absence of affinity-purified guinea pig anti-TARC poly-
gration of Hut78 cells toward TARC (50 ng/ml) was assessed in the

Each point represents mean

B.pertussis

were pretreated without or with indicated concentration of

signal transduction may be independent of Ca²⁺

too low to detect elevation of cytosolic calcium in restricted

gene expression has been shown to cause chemotaxis. Our recent studies also showed little specific binding on

PBMC. By using one of the trapped signal sequences as a

trap using an Epstein-Barr virus trapping vector, pDREF-

selectively cloning cDNAs for secretory molecules and receptors

whether DARC mediates the effect of TARC on these cells.

Recently, DARC has been shown to be expressed on endothelial
cells lining postcapillary venules (51, 52). It remains to be seen
whether DARC mediates the effect of TARC on these cells.

Consistent to little specific binding, TARC did not induce
chemotactic response or Ca²⁺ mobilization in monocytes or
granulocytes at all. On the other hand, TARC clearly induced
chemotaxis in two human T cell lines, Hut78 and Hut102 (Fig.
9). TARC, however, had little significant effect on the migration
of peripheral blood resting or activated T cells above back-
ground levels in spite of considerable levels of specific binding
sites for TARC (Fig. 6). The possibility that activated T cells
were desensitized to TARC by an autocrine or paracrine mech-
anism was excluded because T cells do not produce TARC (see
below) and the chemotactic responses using T cells that were
activated in the absence of other accessory cells. First of all, our
chemotactic assay for normal T cells probably needs further
improvements. We should mention that even RANTES that
was used as a positive control had trouble in demonstrating
significant migration of normal T cells above high levels of
background in our assay. It is, nevertheless, noteworthy that
substantial levels of TARC binding were observed only in some
of the T cell lines examined (Fig. 6). Thus, TARC receptor may
be expressed only in a particular subset of normal T cells.
In addition, the status of activation may affect post-receptor sig-
naling events. Hut78 and Hut102 are cutaneous T cell lym-
phoma lines having unique properties of mature and activated
T cells. It remains to be seen whether phenotypic heterogeneity
of T cells such as CD4⁺ versus CD8⁺, naive versus memory,
resting versus activated, and Th subtypes critically affect re-
sponses of normal T cells to TARC.

TARC is induced in PBMC by PHA-stimulation (Fig. 3). It
remains to be determined which types of cells produce TARC
and whether induction occurs directly by mitogenic stimulation
or secondarily by secreted cytokines. In this context, TARC was
not induced in Jurkat by PHA-treatment nor in U937 by treat-
ment with interferon-γ, tumor necrosis factor-α, IL-1α, or IL-
4.2 Similarly, murine TARC was not induced in mouse thymo-
cytes or splenocytes by stimulation with ConA, anti-Thy1, anti-
CD3, or PHA/PMA.3 Interestingly, however, our recent results
showed that TARC was strongly induced in monocytes by cy-
tokines which are known to be produced by Th2 type T cells, suggesting that TARC may play roles in humoral immunity. 4 TARC is also constitutively and selectively expressed in thymus (Fig. 3). For comparison, we examined expression of other chemokines in the same multiple tissue Northern blots. RANTES was expressed strongly in PBL, spleen, and small intestine. MIP-1α was expressed strongly in spleen and PBL. MCP-1 was expressed strongly in most tissues except for PBL and brain. IP-10 was detected in spleen, thymus, PBL, and lung at low levels (Fig. 3). Gattass et al. (53) reported constitutive expression of murine IP-10 in lymphoid organs (thymus, lymph, and lymph nodes) as well as in liver. Thymic and splenic stromal cells were found to produce IP-10. Our recent studies have revealed that murine TARC is expressed in thymic stromal cells. 5

In addition to leukocyte trafficking, some chemokines probably play important roles in the regulation of hematopoiesis and myelopoiesis. Several chemokines have been shown to have inhibitory or stimulatory effects on the proliferation of hematopoietic stem cells or myeloid progenitor cells (54–57). Mice lacking the IL-8 receptor homologue exhibited abnormal expansion of neutrophils and B lymphocytes, suggesting a role of this receptor in the regulation of expansion and development of neutrophils and B cells as well as in chemotaxis of neutrophils (58). A murine CXC chemokine PBSF/SDF-1α was shown to be produced by bone marrow stromal cell lines and to augment growth of pre-B cells in the presence of IL-7 (30, 59). The constitutive and selective expression of TARC in thymus suggests that TARC is surface involved in the function of thymus such as T cell development or homing. It remains to be seen whether TARC acts on any thymocytes or only on particular subsets of thymocytes. Isolation of the mouse homologue of TARC and production of the recombinant murine protein now in progress will help us answer these questions. Generation of TARC-knockout mice will also be useful for elucidation of physiological functions of TARC.

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REFERENCES

1. Baggiolini, M., Dewald, B., and Moser, P. (1994) Adv. Immunol. 55, 97–179.
2. Oppenheim, J. J., Zachariae, C. O., Mukaida, N., and Matsushima, K. (1991) Annu. Rev. Immunol. 9, 617–648.
3. Matsushima, K., Morishita, K., Yoshimura, T., Lavu, S., Kobayashi, Y., Liew, W., Appella, E., Kung, H. F., Leonard, E. J., and Oppenheim, J. J. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 9704–9708.
4. Miller, M. D., Hata, S., De Waal Malefyt, R., and Kranz, M. S. (1989) J. Immunol. 143, 2907–2916.
5. Kitaoka, M., Kajikawa, T., Imai, T., Harada, S., Comolandi, C., Tiffany, H. L., Murphy, P. M., and Yoshie, O. (1996) J. Biol. Chem. 271, 7725–7730.

4 T. Imai, M. Kakizaki, M. Nishimura, and O. Yoshie, manuscript in preparation.

12. Oppenheim, J. J., Zachariae, C. O., Mukaida, N., and Matsushima, K. (1991) Annu. Rev. Immunol. 9, 388–397.
13. Luster, A. D., Unkeless, J. C., and Ravetch, J. V. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 415–425.
14. Baggiolini, M., and Dahinden, C. A. (1991) Immunol. Today 15, 127–133.
15. Carr, M. W., Roth, S. J., Luther, E., Rose, S. S., and Springer, T. A. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 5359–5363.
16. Schall, T. J., Bacon, K., Toy, K. J., and Goeddel, D. V. (1990) Nature 347, 669–671.
17. Tanaka, Y., Adams, D. H., Hubshers, S., Hirano, H., Siebenlist, U., and Shaw, S. (1993) Nature 361, 79–82.
18. Taub, D. D., Conlon, K., Lloyd, A. R., Oppenheim, J. J., and Kelvin, D. J. (1993) Science 260, 235–238.
19. Keiner, G. S., Kennedy, J., Bacon, K. B., Kleyensteuber, S., Largesaepda, D. A., enkins, N. A., Copeland, N. G. B., Bazan, J. F., Moore, K. W., Schall, T. J., and Zlotnik, A. (1994) Science 266, 1395–1399.
20. Yoshida, T., Imai, T., Maiko, M., Nishimura, M., and Yoshie, O. (1995) FEMS Lett. 360, 155–159.
21. Murphy, P. M. (1994) Annu. Rev. Immunol. 12, 593–633.
22. Gattass, C. R., Kuhns, D. B., Timb, H. L., Moser, P. M., Li, X., Francke, U., and Murphy, P. M. (1993) J. Exp. Med. 177, 1421–1427.
23. Neete, K., DiGregorio, D., Mak, J. Y., Horuk, R., and Schall, T. J. (1993) Cell 72, 415–425.
24. Chao, I. F., Myers, S. J., Herman, A., Frank, C., Connelly, A. J., and Coughlin, S. R. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 2752–2756.
25. Power, C. A., Meyer, A., Nemet, K., Bacon, K. B., Hoagwood, J., Prouxfoott, E., Dray, J. K., and Wells, T. N. C. (1990) J. Biol. Chem. 270, 1495–1500.
26. Combsi, C., Ajioka, S. K., and Murphy, P. M. (1991) J. Biol. Chem. 266, 16491–16494.
27. Neete, K., Darbonne, W., Oges, J., Horuk, R., and Schall, T. J. (1993) J. Biol. Chem. 268, 12247–12249.
28. Chauhduri, A., Bzreka, V., Poljokova, J., Pogo, A. O., Hesselgesser, J., and Horuk, R. (1994) J. Biol. Chem. 269, 7835–7838.
29. Horuk, R., Wang, Z., Peper, S. C., and Hesselgesser, J. (1994) J. Biol. Chem. 269, 17730–17733.
30. Tashiro, K., Tada, H., Heliker, R., Shirouzu, M., Nakano, T. and Honjo, T. (1993) Science 260, 600–607.
31. TARC-knockout mice will also be useful for elucidation of physiologival functions of TARC.
Molecular Cloning of a Novel T Cell-directed CC Chemokine Expressed in Thymus by Signal Sequence Trap Using Epstein-Barr Virus Vector

Toshio Imai, Tetsuya Yoshida, Masataka Baba, Miyuki Nishimura, Mayumi Kakizaki and Osamu Yoshie

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