Molecular Cloning and Functional Characterization of a Novel CC Chemokine, Stimulated T Cell Chemotactic Protein (STCP-1) That Specifically Acts on Activated T Lymphocytes*

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A novel human chemokine STCP-1 (stimulated T cell chemotactic protein) was isolated from an activated macrophage cDNA library. The chemokine has four cysteine positions in a manner that identifies STCP-1 as a member of the CC chemokine family. The amino acid sequence shows 34% identity with RANTES. The gene consists of 3 exons and 2 introns with the position of intron/exon boundaries similar to that of RANTES. The gene is expressed as a 3.4-kilobase transcript on lymph node, thymus, and appendix. STCP-1 induces Ca\(^{2+}\) mobilization in a small percentage of primary activated T lymphocytes, but on repeated stimulation the percentage of T lymphocytes that respond to STCP-1 increases. The chemokine STCP-1 does not induce Ca\(^{2+}\) mobilization in monocytes, dendritic cells, neutrophils, eosinophils, lipopolysaccharide-activated B lymphocytes, and freshly isolated resting T lymphocytes. Similarly, STCP-1, while acting as a mild chemoattractant for primary activated T lymphocytes, is a potent chemoattractant for chronically activated T lymphocytes but has no chemoattractant activity for monocytes, neutrophils, eosinophils, and resting T lymphocytes. As STCP-1 acts specifically on activated T lymphocytes, it may play a role in the trafficking of activated/effector T lymphocytes to inflammatory sites and other aspects of activated T lymphocyte physiology.

Chemokines are a large and growing family of 6–14-kDa (nonglycosylated) heparin-binding proteins that mediate a wide range of biological functions (1). The chemokines are divided into two main families based on the position of four cysteine residues that form two disulfide bonds. The presence or absence of an amino acid between the first two conserved cysteine residues distinguishes the CC and CXC chemokines. Generally the CXC family of chemokines acts on neutrophils, while the CC family members act on other leukocytes, which include monocytes, lymphocytes, eosinophils, basophils. Recently a new family of chemokines was defined with the discovery of lymphotactin, which lacks two of the four cysteine residues found in other chemokines characterized to date and acts specifically on lymphocytes (2). The chemokine receptors are divided into the CXC (4 members) and CCR families (5 members) for CXC and CC chemokines, respectively (3). All are 7 transmembrane domain receptors that signal through G proteins. Most chemokine receptors bind to several chemokines, and some chemokines also interact with several receptors.

Chemokines play a vital role in leukocyte adhesion and extravasation. In various in vitro assays, chemokines support the chemotaxis or transendothelial migration of leukocytes (1), while in vivo injection (4, 5) or overexpression of chemokines (6) results in leukocyte accumulation at the site of chemokine expression. Antagonism of chemokine action prevents leukocytes from entering inflammatory sites (7–9) and has beneficial effects in several acute and chronic inflammatory models (10–16). As well as being active in leukocyte adhesion and extravasation, chemokines modulate angiogenesis (17–20), hematopoiesis (1) and T lymphocyte activation (21, 22). Recently, the CCR-3, CCR-5, and CXCR-4 chemokine receptors were found to act as co-receptors with CD4 for entry of M tropic and Tropic HIV-1 (23, 24).

Activated T lymphocytes have lower costimulation requirements (25) than resting T lymphocytes and show different adhesion and trafficking profiles (26, 27) while they respond to different chemokines (28). We describe here a novel chemokine that acts specifically on activated T lymphocytes. Because the activity profile of this chemokine is restricted to activated T lymphocytes, we called it STCP-1 (stimulated T cell chemotactic protein).

MATERIALS AND METHODS

Generation and Activation of Human Macrophages—Macrophages were generated in in vitro differentiation from monocytes. Peripheral blood mononuclear cells were prepared from leukopheresis packs by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density centrifugation. Monocytes were allowed to adhere for 2 h at 37 °C, after which time the nonadherent cells were removed by two washes with warm medium. Each flask then received 40 ml of RPMI medium 1640 containing 10% human AB serum (Gemini Bioproducts, Calabasas, CA), 5% fetal calf serum, 50 mm NaCl, 100 units/ml penicillin, and 100 μg/ml streptomycin. The cells were fed with a complete change of fresh media on days 3 and 6. On day 7, the media of each of the 7 flasks was adjusted to contain one of the following 7 stimuli; 1 μg/ml E. coli K562 LPS (Sigma), 100 units/ml IFN-γ, LPS, and IFN-γ in combination, 100 ng/ml tumor necrosis factor in combination with 10 ng/ml IL-1β, 50 ng/ml IL-4, 10 ng/ml IL-10, and 10 ng/ml Consensus-IFN-α. Consensus-IFN-α was prepared at Amgen. All other cytokines were purchased from R&D Systems (Minneapolis, MN). Macrophages were stimulated for 3 h with various stimuli, prior

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The abbreviations used are: HIV, human immunodeficiency virus; LPS, lipopolysaccharide; IFN, interferon; EST, expressed sequence tag; DME, Dulbecco’s modified Eagle’s medium; FCS, fetal calf serum; OVA, ovalbumin; mAb, monoclonal antibody; IL, interleukin; PBS, phosphate-buffered saline; DPBS, Dulbecco’s phosphate-buffered saline; ICAM-1, intercellular adhesion molecule 1; RANTES, regulated on activation normal T cell expressed and secreted; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; kb, kilobase(s).

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to isolation of the RNA. Purity and differentiation of 7-day macrophages were determined in separate cultures by flow cytometric analysis. More than 90% of the cells were positive for both CD14 and CD71.

Construction of cDNA Library and Isolation of cDNA and Genomic Clone—Cells from various treatments were combined, and poly(A) RNA was isolated by using the Invitrogen Fast Track mRNA isolation kit (Invitrogen Corp., San Diego, CA). A cDNA library was constructed in the pcDNAII vector (Invitrogen). The library was screened in EST analysis (29). Individual clones from this library were randomly picked and sequenced on an Applied Biosystems 373A automated DNA sequencer using vector primer and Taq dye-terminator reactions (Applied Biosystems). The resulting nucleotide sequences obtained from the randomly picked clone STCP-1 was translated, then compared with the existing data base of known protein sequences using a modified version of the FASTA program (30). The human genomic clone was isolated from a human genomic P1 library (Genome System Inc., St. Louis, MO) with a labeled human cDNA probe.

Expression of the Protein in E. coli—The STCP-1 cDNA sequence coding for the protein from amino acid 25 (Gly) to amino acid 93 (Gln) was cloned into a prokaryotic expression vector and transformed into Escherichia coli. The 8-kDa protein was purified from E. coli and used in all the biological function analyses.

Northern Blot Analysis—Northern blots of multiple human tissues were purchased from CLONTECH (Palo Alto, CA). In addition, equal amounts of poly(A) mRNA isolated from resting monocytes, CD4 T cells, macrophages, LPS-stimulated monocytes, stimulated macrophages (as described above), and chronically activated CD4 T cells (as described below) was blotted onto nitrocellulose. The Northern blots were probed with a human STCP-1 cDNA probe.

Purification of Cell Populations—Human peripheral blood was collected in 10% (v/v) 0.1 mM EDTA, layered onto 1-Step Polymorphs gradient (Accurate Chemical Co., Westbury, NY), and centrifuged at 400 g for 30 min at room temperature. Neutrophil and mononuclear cell layers were collected, resuspended in DPBS without calcium and magnesium (Life Technologies, Inc.), and centrifuged for 15 min at ~750 × g. Red blood cells were lysed in the neutrophil fraction by resuspending the pellet in E-Lyse (Cardinal Associates, Santa Fe, NM) for 5 min on ice. Both cell fractions were washed twice with ice-cold DPBS. The mononuclear cell layers were collected, rinsed, centrifuged, and resuspended in DPBS without calcium and magnesium for 2–3 h, and then nonadherent cells were gently washed off the plate. After a further 12 h the nonadherent dendritic cells were washed off the plate and depleted with anti-CD19 and anti-CD2 Dynabeads (5 beads/cell), followed by culture in 50 ng/ml granulocyte-macrophage colony-stimulating factor and 40 ng/ml IL-4, DMEM, 10% FCS plus additives (as described above), and chronically activated CD4 T lymphocytes were isolated and stimulated with OVA (50 μg/ml) in the presence of mitomycin-treated splenocytes followed by 4–5 days in

FIG. 2A, comparison of amino acid sequences between STCP-1 and RANTES. Identical amino acid sequences are indicated by |. Similar amino acid sequence are indicated by . The locations of the intron are shown by arrows. The four conserved cysteines in bold type, B, sequences of the intron-exon junctions of the human STCP-1 gene. Exon sequences are shown in uppercase letters, and intron sequences are shown in lowercase letters. The length of each intron is shown in parentheses.
IL-2-containing media. The OVA-specific T cells were then either used or re-stimulated for 5 days and expanded in IL-2 before use in assays.

**Antibodies and Reagents**—The following mAbs were used in this study: OKT3, an anti-human CD3 mAb (ATCC); 145–2C11, an anti-mouse CD3 mAb (ATCC), anti-human CD28 mAb (Becton Dickinson), and anti-mouse CD28 mAb (Pharmingen). All chemokines with the exception of STCP-1 were purchased from R&D, Minneapolis. Granulocyte-macrophage colony-stimulating factor and IL-4 were also purchased from R&D. mAbs FD411.8 (rat anti-mouse LFA-1), YN1.7 (rat anti-mouse ICAM-1), PS/2 (rat anti-mouse CD49d) were obtained from the ATCC whereas 2H8 (hamster anti-mouse CD31) was obtained from Endogen, and MEC13.3 and 390 (rat anti-mouse CD31) mAbs were obtained from Pharmingen. The polyclonal to STCP-1 was generated by three intradermal injections of STCP-1 coupled to keyhole limpet hemocyanin in Titermax at several sites on the back of a rabbit at 3-week intervals. The rabbits were bled via the ear 10–20 days after the final immunization, and the polyclonal was purified by Protein A affinity chromatography. The human endothelial cell line ECV304 was purchased from ATCC, while the bEND3 cell line (31) was kindly provided by Dr. W. Risau (Max-Planck-Institute, Bad Neuheim, Germany).

**Preparation of Chronically Activated T Lymphocytes**—As described previously (32) six-well Falcon plates were coated overnight with 10 µg/ml anti-CD28 and 2 µg/ml OKT3 and then washed twice with PBS. Umbilical cord blood CD4 lymphocytes (Poietic Systems, German Town) or adult blood T lymphocytes were then cultured at 10^5–10^6 cells/ml in DMEM with 10% FCS and IL-2 (4 ng/ml). After 5 days, the activated T lymphocytes were washed once in DMEM and recultured for 4–7 days in DMEM with 10% FCS and IL-2 (1 ng/ml). Following this, the activated T lymphocytes were re-stimulated for 5 days with anti-CD28/OKT3/IL-2 and then cultured again with IL-2 for 4 days. Activated T lymphocytes were maintained in this way for a maximum of three cycles. Activated mouse T cells were prepared in a similar manner but using anti-mouse CD3 and anti-mouse CD28 mAbs, while using human IL-2 in the rest cycle.

**ECV304/bEND3 Transmigration Assay and Chemotaxis Assays**—The inserts of Costar Transwells of 5-µm pore diameter were coated with 2% gelatin for 2 h. Then 0.45 ml of DMEM with 5% FCS was placed in the lower wells of the chambers, and 2 x 10^5 ECV304 cells or bEND3 cells were added to each gelatin-coated insert in 0.2 ml of DMEM, 5% FCS. After 2 days the wells and inserts were washed twice with RPMI with 0.5% human serum albumin, 10 mM Hepes, and chemokine was added to the lower well. The cells under study were washed once in RPMI and resuspended at 4 x 10^5 cells/ml in RPMI with 0.5% human serum albumin and 10 mM Hepes. An aliquot of 100 µl of cell suspension was added to each insert. After 2 to 4 h the inserts were removed, and the number of cells which had migrated through the ECV304 monolayer to the lower well counted for 60 s with the FACScan set to count for 60 s. Chemotaxis assays were identical to ECV304 migration assays, but fibronectin-coated inserts (10 µg/ml) were used instead of inserts coated with a monolayer of ECV304 cells. In all cases the data points were the result of duplicate wells, with the mean value shown and the error bars representing the sample standard deviation.

**Ca^{2+} Mobilization Assay**—Two methods were used. To analyze Ca^{2+} mobilization in subsets of cells lymphocytes were washed twice in PBS and resuspended in warmed PBS containing 1 mM MgCl_2, 1 mM CaCl_2, 5 µM Fluo 3/AM cell permeant (Molecular Probes, Catalog No. F1242). The cells were then incubated at 37 °C for 30 min. Following this the labeled cells were washed twice in ice-cold PBS with 1 mM MgCl_2 and 1 mM CaCl_2 and resuspended at 10^8 cells/ml in DPBS containing 1 mM CaCl_2, 0.5 mM MgCl_2, 10 mM Hepes, and 5.5 mM glucose. An aliquot of 0.4 ml was taken and placed on the FACScan with the gates set to acquire the cells of interest examining FL1 (fluorescein...
FIG. 4

**A** Neutrophils

| Treatment   | Mean No of Cells Migrated |
|-------------|---------------------------|
| STCP-1 10 ng/ml | 10000                    |
| STCP-1 100 ng/ml| 10000                   |
| Rantes 10 ng/ml  | 10000                   |
| Rantes 100 ng/ml | 10000                  |
| IL8 10 ng/ml   | 10000                   |
| IL8 100 ng/ml  | 10000                   |
| Csa 20 mM    | 10000                   |

**B** Monocytes

| Treatment   | Mean No of Cells Migrated |
|-------------|---------------------------|
| STCP-1 2 ng/ml | 1000                   |
| STCP-1 20 ng/ml| 1000                   |
| STCP-1 200 ng/ml| 1000                  |
| Rantes 100 ng/ml | 1000                 |
| IL8          | 1000                     |

**C** Eosinophils

| Treatment   | Mean No of Cells Migrated |
|-------------|---------------------------|
| STCP-1 1 μg/ml | 1000                |
| Rantes 5 μg/ml | 1000               |
| Rantes 10 ng/ml | 1000              |
| Eosatin 5 μg/ml | 1000              |

**D** Primary Activated CD4 lymphocytes

| Treatment   | Mean No of Cells Migrated |
|-------------|---------------------------|
| Eosatin 100 ng/ml | 1000          |
| Rantes 100 ng/ml  | 1000          |
| STCP-1 0.1 ng/ml  | 1000          |
| STCP-1 1 ng/ml    | 1000          |
| STCP-1 10 ng/ml   | 1000          |
| STCP-1 100 ng/ml  | 1000          |

**E** Activated CD4 lymphocytes (3rd Activation)

| Treatment   | Mean No of Cells Migrated |
|-------------|---------------------------|
| Eosatin 100 ng/ml | 1000          |
| Rantes 100 ng/ml  | 1000          |
| STCP-1 0.1 ng/ml  | 1000          |
| STCP-1 1 ng/ml    | 1000          |
| STCP-1 10 ng/ml   | 1000          |
| STCP-1 100 ng/ml  | 1000          |

**F** Transendothelial Migration of CD4 and CD8 Lymphocytes

| Treatment   | Mean No of Migrated Cells |
|-------------|---------------------------|
| MCP-1       | 1000                      |
| STCP-1      | 1000                      |
| Eosatin     | 1000                      |
| Rantes      | 1000                      |

**G** Phenotype of Umbilical CD4 lymphocytes and Chronic (2nd activation) CD4 Lymphocytes

| Cell Surface Protein | Mean Fluorescent Intensity |
|----------------------|---------------------------|
| CD38                 | 1000                      |
| CD45RO               | 1000                      |
| CD45PA               | 1000                      |
| CD19                 | 1000                      |

**FIG. 4**
RESULTS

Isolation and Characterization of STCP-1 cDNA—While sequencing the stimulated macrophage cDNA library by EST analysis, we identified a 1-kb fragment of cDNA, STCP-1, encoding a protein homologous to the human RANTES. As shown in Fig. 1, it encodes a protein of 93 amino acids. Hydrophobic analysis predicts a hydrophobic signal peptide of 24 amino acids. Beginning with Gly (residue 25), the mature protein containing 69 amino acids has a predicted molecular mass of 8 kDa. No apparent sites for N-linked glycosylation are present. The protein contains four cysteines whose positions are identical to that of RANTES (Fig. 2A), suggesting that STCP-1 belongs to the C-C family of the chemokines. The amino acid sequences of the protein share 62% similarity and 34% identity with that of RANTES.

Genomic Structure of the Human STCP-1 Gene—The human STCP-1 genomic clone was isolated from a P1 genomic library by using a labeled human cDNA probe. About 8 kb of the gene has been sequenced. The gene consists of 3 exons and 2 introns. The sequences of the intron-exon junctions conform to the consensus sequence of eukaryotic splice junctions (Fig. 2B). The genomic structure of the STCP-1 shares great homology with RANTES in the sizes of exons and the location of the intron-exon junction.

Tissue and Cell-specific Expression of STCP-1—We used a 282-base pair cDNA fragment spanning the coding region of STCP-1 as a probe to analyze the tissues and cell type-specific expression of the STCP-1 gene. As shown in Fig. 3A, STCP-1 was expressed as a 3.4-kb transcript in the tissues of thymus, lymph node, and appendix. The STCP-1 cDNA clone was originally isolated from the stimulated macrophage cDNA library. In a preliminary Northern blot analysis, STCP-1 transcripts were also found in activated monocytes, resting and activated macrophages, but not in resting or activated CD4 lymphocytes (Fig. 3B).

Expression of Protein in E. coli—To express the recombinant protein in E. coli, we constructed an expression vector which contains a coding region from residue 25 (Gly) to residue 93 (Gln) downstream of the luciferase promoter. The protein expressed from E. coli was purified and analyzed. The protein has a molecular mass of 8 kDa as we predicted from amino acid sequence (data not shown).

STCP-1 Is a Chemoattractant for Activated T Lymphocytes—To search for a responsive cell population to STCP-1, we examined the ability of STCP-1 to support the transendothelial migration of different cell populations. This assay was used in preference to chemotaxis, as the assay has a much lower background and is therefore more sensitive and robust. STCP-1 was unable to support the transmigration of either neutrophils, monocytes, or eosinophils (Fig. 4, A, B, and C) across a monolayer of ECV304 cells (an endothelial cell line) at several concentrations. Other chemokines reported to act on these cell populations were able to direct migration of these different cell types. However, when resting T lymphocytes from umbilical blood were activated with anti-CD28 and anti-CD3 mAbs followed by culture in IL-2 for 5 days, these activated T lymphocytes became responsive to STCP-1 (Fig. 4D). While the transendothelial migration of activated T lymphocytes supported by STCP-1 was not as great at that directed by RANTES, a further two rounds of stimulation of T lymphocytes resulted in increased responsiveness to STCP-1 (Fig. 4E). A polyclonal antibody raised against STCP-1 blocked STCP-1-induced transendothelial cell migration of these chronically activated T lymphocytes (Fig. 4E). Chronically activated adult CD4 and CD8 lymphocytes chemotaxed to STCP-1 indicating that both CD4 and CD8 T lymphocyte subsets are responsive to STCP-1 (Fig. 4F). Checkerboard analysis demonstrated that STCP-1 stimulated chemotaxis of chronically activated T lymphocytes rather than random migration in the transendothelial chemotaxis assays used here (Table I).

To ensure that the in vitro culture system used in this study was generating chronically activated T lymphocytes we stained umbilical T lymphocytes and secondary activated T lymphocytes with mAbs reacting with surface molecules which define activation markers. On T lymphocyte activation, CD31, CD45RA, and CD62L are down-regulated from the T lymphocyte surface, while CD45RO expression is up-regulated on T lymphocytes. As shown in Fig. 4G repeated activation of umbilical T lymphocytes resulted in a transition to a phenotype predictive of activated T lymphocytes. By the second stimulation the chronically activated T lymphocytes were CD45RA-negative, CD62L-negative, CD31-negative, and CD45RO-positive as expected.

STCP-1 activity is not restricted to the human system, as STCP-1 was also found to be a very potent chemoattractant for the chronically activated mouse T lymphocytes, as was mouse MIP-α, MIP-1β, and MCP-1 (Fig. 5A). STCP-1-directed transendothelial migration was blocked by pretreatment of the chronically activated T lymphocyte cells with anti-CD11a and anti-ICAM-1 mAbs (Fig. 5B) and partially with anti-CD49d mAbs, while anti-CD31 mAbs had no effect. As in the human system both chronically activated CD4 and CD8 lymphocytes were responsive to STCP-1 (Fig. 5C). To examine T lymphocytes activated in a more physiological manner mice were immunized in the footpad with OVA, and a primary T lymphocyte
FIG. 5. **STCP-1 is a chemoattractant for activated mouse T lymphocytes.** A, secondary activated mouse T lymphocytes were tested for their ability to transmigrate across bEND3 cells coated on 5.0 μM Costar Inserts to various concentrations of STCP-1, using mouse MCP-1, MIP-1α, and MIP-1β as a positive control. Background migration to media or 100 ng/ml TCA3 was 938 ± 67 and 967 ± 54. We also tested various anti-adhesion mAbs L (FD411.8, anti-LFA-1), P (PS/2, anti-CD49d), I (YN1.7, anti-CD54) CD31s (combination of 2H8, MEC 13.3, and 390) for their effect on STCP-1-induced transendothelial migration (B). In C secondary activated mouse CD4 and CD8 lymphocytes were tested for chemotaxis to STCP-1, MIP-1α, MIP-1β, MCP-1, TCA3, and C10 (all at 100 ng/ml). STCP-1 was also tested for its ability to direct transendothelial migration of an activated primary T cell line to OVA, using MCP-1 at 100 ng/ml as a positive control (D). MIP-1α, MIP-1β, MCP-5, and TCA3 were also tested, again at 100 ng/ml. Finally, secondary activated T lymphocytes (E) were examined for their ability to chemotax across fibronectin-coated 5.0 μM Costar Inserts to various concentrations of STCP-1. These experiments were performed twice with similar results, and data points represent the mean of two wells with the error bars representing the sample standard deviation of these two samples.
STCP-1 induces Ca^{2+} mobilization in activated T lymphocytes. Monocytes (A), dendritic cells (B), neutrophils (C), eosinophils (D), LPS-activated B cells (E), and chronically activated umbilical blood CD4 lymphocytes (F) were labeled with Fura 2 and tested for their response to STCP-1 in terms of Ca^{2+} mobilization (solid lines). We used the following controls at 100 ng/ml (short dashed lines): MCP-1 for monocytes, RANTES for dendritic cells, IL-8 for neutrophils, RANTES for eosinophils, and anti-IgG for B lymphocytes which had been activated for 4 days with 10 μg/ml LPS. As STCP-1 activates chronically activated T lymphocytes we show the Ca^{2+} mobilization for a negative control (eotaxin) with these cells. The experiments were performed three times with identical results.

The response to STCP-1 in terms of Ca^{2+} blood CD4 lymphocytes (phils) was examined. However, chronically activated T lymphocytes responded to STCP-1 (Fig. 6). Similarly, previously characterized chemokines MCP-1, MIP-1α, IP-10, and RANTES, which were poor stimulants of primary activated T lymphocytes, proved to be much more potent chemokine agonists for chronically activated T lymphocytes. Other chemokines such as eotaxin (Fig. 7) had no activity either on primary activated or chronically activated T lymphocytes. Therefore, we tested STCP-1 for its ability to support chemotaxis of chronically activated mouse T lymphocytes across fibronectin-coated inserts. STCP-1 supports the chemotaxis of chronically activated T lymphocytes (Fig. 5E).

STCP-1 Induces Ca^{2+} Mobilization in Activated T Lymphocytes—Due to the poor transendothelial migration of primary activated T lymphocytes supported by STCP-1, we thought it possible that only a subpopulation of primary activated T lymphocytes expressed the relevant chemokine receptor for STCP-1. To examine this possibility, we looked at the ability of STCP-1 to stimulate Ca^{2+} mobilization in activated T lymphocytes and other leukocyte subsets. As predicted from our results in the ECV304 transmigration assays, STCP-1 was unable to stimulate Ca^{2+} mobilization in monocytes, neutrophils, eosinophils, or dendritic cells, while the relevant chemokines for each of these cell populations proved stimulatory (Fig. 6, A–D). We also examined the response of LPS-activated B lymphocytes to STCP-1 to determine whether activation of other lymphocyte subsets resulted in responsiveness to STCP-1. While cross-linking of surface IgG on activated B lymphocytes induced Ca^{2+} mobilization, STCP-1 had no effect (Fig. 6E).

However, chronically activated T lymphocytes responded to STCP-1 (Fig. 6F). When we examined Ca^{2+} mobilization in primary and chronically activated T lymphocytes to STCP-1, using a FACScan, which can detect Ca^{2+} mobilization in subsets of cells at a much larger proportion of the chronically stimulated T lymphocytes than the primary activated T lymphocytes responded (Fig. 7). Similarly, previously characterized chemokines MCP-1, MIP-1α, IP-10, and RANTES, which were poor stimulants of primary activated T lymphocytes, proved to be much more potent chemokine agonists for chronically activated T lymphocytes. Other chemokines such as eotaxin (Fig. 7) had no activity either on primary activated or chronically activated T lymphocytes.
STCP-1, a Novel Chemokine for Activated T Lymphocytes

**DISCUSSION**

In this study we describe a novel human chemokine, STCP-1, which shares homology with RANTES in the amino acid sequence and the genomic structure. The gene is specifically expressed on the lymph node thymus and appendix. STCP-1 is selectively chemotactic for activated T lymphocytes. Numerous chemokines act as chemotactic attractants for lymphocytes, although the data from various groups are contradictory. Several studies have demonstrated that certain CXC (33, 34) and CC chemokines (35, 36) direct the transendothelial migration or chemotaxis of memory T lymphocytes, while other reports claim that activation of T lymphocytes (28, 37) is required for the response to several chemokines. These discrepancies probably reflect the different signal/noise ratios of the assays used by individual groups and/or the different culture condition used. In this study, STCP-1 did not act on monocytes, neutrophils, eosinophils, LPS-activated B lymphocytes, or freshly isolated resting T lymphocytes. However, upon activation of T lymphocytes by culture with plate-bound anti-CD28/CD3 mAbs in the presence of IL-2, followed by culture in IL-2 for 5 days, STCP-1 was found to act as a weak chemotactic attractant for these cells. Similarly, STCP-1 was able to stimulate Ca$^{2+}$ mobilization in a subset of activated T lymphocytes. MCP-1, IP-10, MIP-1$\alpha$, and RANTES also stimulated Ca$^{2+}$ mobilization in a subset of activated T lymphocytes, while eotaxin was inactive. Further rounds of stimulation of the T lymphocytes resulted in increased chemotaxis of the T lymphocytes to STCP-1 as well as other chemokines. Ca$^{2+}$ mobilization data also show that a much greater percentage of chronically stimulated T lymphocytes respond to STCP-1, MCP-1, IP-10, MIP-1$\alpha$, and RANTES than was observed with the primary activated T lymphocytes. Therefore, repeated rounds of stimulation increase the percentage of T lymphocytes which respond to these CC and CXC chemokines.

STCP-1 is unusual in only acting on activated T lymphocytes. Two CXC chemokines, Mig and IP-10, were also recently reported to act on activated T lymphocytes as well as monocytes (38). We feel it unlikely that the receptor for STCP-1 is the Mig/IP-10 chemokine receptor, CXCR-3, as there is as yet no example of a CC chemokine which binds to a CXC chemokine receptor. Also, IP-10 did not desensitize activated T cells to stimulation with STCP-1. TER1 (39) could possibly be a receptor for STCP-1, as this receptor is weakly expressed on peripheral blood lymphocytes. However, the receptor is not up-regulated on lymphocyte activation and so it would appear unlikely. Finally, a recently described chemokine, MIP-3$\beta$ (40), shows an identical distribution to STCP-1, as it is expressed selectively in appendix, lymph nodes, and thymus, as is STCP-1. The function for this chemokine is not yet known, but it is likely that MIP-3$\beta$ may, like STCP-1, act selectively on activated T lymphocytes. The lack of expression of STCP-1 in spleen and lung, where macrophages are also found, is surprising. This may reflect selective expression of STCP-1 by subsets of macrophages. Future studies with a two-site enzyme-linked immunosorbent assay will examine the expression of STCP-1 by various leukocyte subsets in more detail.

While it is known that subsets of memory T lymphocytes respond to certain chemokines (33), the physiological reason for this is not yet known. However, just as the expression of certain cell adhesion molecules directs activated T lymphocytes to inflammatory sites it is also possible that the expression of specific chemokine receptors on activated T lymphocytes is also required for their trafficking to these sites. This study demonstrates that on repeated stimulation, T lymphocytes up-regulate chemokine receptors for chemokines which are known to be expressed in acute and chronic inflammatory sites, such as MIP-1$\alpha$ and MCP-1. In addition T lymphocyte activation probably also results in the expression of novel chemokine receptors which are selectively expressed on these cells and which may play a unique role in the adhesion and transendothelial migration of activated T lymphocytes at inflammatory sites. STCP-1, which acts specifically on activated T lymphocytes, may bind to one such chemokine receptor.

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