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Structure and expression of two highly related genes encoding SCM-1/human lymphotactin

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Abstract SCM-1/lymphotactin is a chemokine-like molecule produced selectively, if not exclusively, by activated CD8$^+$ T cells. Here we report that there are two highly homologous SCM-1 genes, which we designate as *SCM-1α* and *SCM-1β*. Both genes have three exons and two introns. The 1st intron of *SCM-1α* contains a pseudogene of the ribosomal large subunit L7a. In *SCM-1β*, a 1.5-kb region including about a quarter of the *L7a* pseudogene is deleted from the 1st intron. Otherwise, the two genes are highly homologous including the 5′ and 3′ flanking regions. Both genes were mapped to human chromosome 1q23. The two genes were similarly induced in peripheral blood mononuclear cells by mitogenic stimulation. Primer extension and RNase protection revealed several transcription initiation sites. The biological activities of SCM-1α and SCM-1β, which have two amino acid differences at positions 7 and 8 in the mature proteins, remain to be compared.

Key words: Chemokine; Gene duplication; Chromosome mapping; Gene expression; Transcriptional initiation site

1. Introduction

The tissue accumulation of various leukocytes is a major sign of inflammatory responses. It is now known that structurally related 8–10-kDa heparin-binding polypeptides, collectively called chemokines, are secreted from various types of cells upon proinflammatory stimulation and play roles in recruitment and activation of different subsets of leukocytes [1,2]. Chemokines are currently divided into two subfamilies, the CXC (or α) and CC (or β) ones, depending on whether the first two conserved cysteines in the mature peptide sequences are separated by a single amino acid or adjacent. The two subfamilies also differ in their target cell selectivity; the CXC chemokines are mainly chemotactic for neutrophils, whereas the CC chemokines are mainly chemotactic for monocytes and also for basophils, eosinophils, and lymphocytes with variable selectivity. The genes for the CXC chemokines and those for the CC chemokines are separately clustered on human chromosome 4q21–q23 and 17q11–q21, respectively [1,2].

Previously, we identified a novel secretory protein from a library of human phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC) by using an efficient signal sequence trap based on an Epstein-Barr Virus vector [3]. The molecule, which we designated as single cysteine motif-1 (SCM-1), is significantly related to chemokines, especially to the CC subfamily, but retains only the 2nd and the 4th of the four cysteines conserved in other chemokines. SCM-1 also carries a C-terminal sequence 20–30 amino acids longer than those of other chemokines. Its gene segregated with human chromosome 1 in somatic hybrid cell lines [3]. Furthermore, SCM-1 was found to have a strong sequence similarity to a newly described murine protein lymphotactin (60.5% identity at the amino acid level) [4]. Lymphotactin was isolated from a murine pro-T cell cDNA library, found to be induced in CD8$^+$ T cells and CD4$^-$CD8$^-$ thymocytes upon mitogenic stimulations, and shown to be chemotactic for lymphocytes but not for monocytes or granulocytes [4]. Subsequently, the human homologue of lymphotactin was isolated and identical to SCM-1 [5]. Human lymphotactin was shown to be produced mainly by activated CD8$^+$ T cells and to induce chemotactic responses and Ca^{2+} flux in lymphocytes but not in monocytes [5].

As a first step toward gaining an understanding of molecular mechanisms regulating the SCM-1 gene expression, we isolated genomic clones hybridizing with the SCM-1 cDNA. Unexpectedly, we found two highly homologous SCM-1 genes. These genes, termed *SCM-1α* and *SCM-1β*, were both mapped to human chromosome 1q23 and similarly induced in PBMC by mitogenic stimulation.

2. Materials and methods

2.1. Cells

Various human lymphoid cell lines and HeLa were routinely maintained in our laboratory [6]. PBMC were isolated from heparinized venous blood from healthy adult donors using Ficoll-Paque (Pharmacia, Uppsala, Sweden). Cells were stimulated with 1/100 PHA (Life Technologies, Inc., Grand Island, NY), 50 ng/ml of PMA (Sigma, St. Louis, MO), or anti-CD3 antibody preadsorbed onto plastic dishes.

2.2. Blot analyses

Southern blot analysis was carried out essentially as described previously [7]. Briefly, genomic DNA was digested with indicated restriction enzymes, gel-fractionated, and blotted onto a Hybond N+ filter membrane (Amersham Japan, Tokyo). Filters were prehybridized in a solution of 5×SSPE, 10×Denhardt's solution, 50% formamide, 2% SDS and 100 μg/ml of salmon sperm DNA at 42°C for 1 h and hybridized overnight in the same buffer containing the SCM-1 cDNA labeled with [^{32}P]dCTP using a Multiprime DNA Labeling System (Amersham Japan). After washing at 60°C in 0.1×SSC and 0.1% SDS, filters were subjected to autoradiography. Filters for human allele frequency determination were purchased from BIOS Laboratories (New Haven, CT). Northern blot analysis was carried out as described previously [3].

2.3. Isolation and sequencing of the genomic clones

A human whole blood genomic library in Lambda Dash II was purchased from Stratagene (La Jolla, CA). About 10^6 plaques were prehybridized in a solution of 5×SSC, 5×Denhardt's solution, 50% formamide, 0.5% SDS and 100 μg/ml of salmon sperm DNA at 42°C for 1 h and hybridized overnight in the same buffer containing the

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^{32}P-labeled SCM-1 cDNA. After washing at 60°C in 0.1×SSC and 0.1% SDS, plaques that were positive on duplicate filters were purified. Seven independent clones were isolated. Inserts were mapped by digestion with restriction enzymes and hybridization with the SCM-1 cDNA. Two clones contained a 5.3-kb *Eco*RI fragment and five clones contained a 3.8-kb *Eco*RI fragment, both hybridizing with the SCM-1 cDNA. The 5.3-kb *Eco*RI fragment from clone hg40 and the 3.8-kb *Eco*RI fragment from clone hg44 were subcloned into pBluescript II (Stratagene), and sequential deletions were generated using Deletion kit (Takara Shuzo, Kyoto, Japan). Nucleotide sequences were determined using AutoRead Sequencing kit and A.L.F. DNA Sequencer (Pharmacia). The exons, exon-intron junctions, 5′ flanking sequences, and 3′ flanking sequences were determined on both strands.

2.4. Chromosomal in situ hybridization

This was carried out essentially as described previously [8]. The 5.3-kb *Eco*RI fragment containing the *SCM-1α* gene and the 3.8-kb *Eco*RI fragment containing the *SCM-1β* gene were labeled using biotin-16-dUTP and Nick Translation kit (Boehringer Mannheim), combined with sheared human DNA, and hybridized to bromodeoxyuridine-substituted human metaphase chromosomes derived from PHA-stimulated PBMC. Biotin-labeled DNA was detected by fluorescein isothiocyanate (FITC)-conjugated avidin (5 μg/ml). Chromosomal identification was done by simultaneous staining with 4,6-diamidino-2-phenylindole (DAPI) and propidium iodide (PI), which produced characteristic low contrast G-like patterns and high contrast R-like patterns, respectively. The hybridizing loci were determined by the FITC and PI images.

2.5. Reverse transcription-polymerase chain reaction

To detect transcripts from the two SCM-1 genes, reverse transcription-polymerase chain reaction (RT-PCR) [9] was carried out using Superscript PreAmplification System (Gibco-BRL, Gaithersburg, MD). First strand cDNAs were synthesized from 0.5 μg of mRNA using oligo(dT) primer. PCR was carried out by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 2 min. In order to normalize cDNA templates, samples in 10-fold dilutions were first amplified with primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (+5′-AGTCAGCCGCATCTTCTTTGC-3′ and −5′-CTCCTGGAAGATGGTGATGGGA-3′). Subsequently, normalized cDNA templates were amplified using primers complementary to both of the SCM-1 nucleotide sequences (+5′-TCAGCCATGAGACTTCTC-3′ and −5′-TAATTTTATTCATGCAGTGCTTTCATA-3′). After amplification, PCR products were purified using a PCR Prep kit (Promega, Madison, WI) and electrophoresed on a 2% GTG agarose gel containing ethidium bromide either directly or after digestion with *Pma*CI (Takara Shuzo). PCR was also carried out using a sense primer specific for the *SCM-1α* nucleotide sequence (+5′-GGGAGTGAAGTCTCAGATAA-3′) or the *SCM-1β* nucleotide sequence (+5′-GGGAGTGAAGTCTCACATAG-3′) and an antisense primer common to both the *SCM-1α* and *SCM-1β* nucleotide sequences (see above).

2.6. Mapping of transcriptional initiation sites

Primer extension analysis was carried out as described previously [7]. In brief, an oligonucleotide primer (5′-CCTTCCACAATGTATGCAGTGAGAGAGCAGATGCC-3′), which was complementary to both *SCM-1α* and *SCM-1β* mRNA, was labeled at the 5′ end with [γ-^{32}P]ATP using T4 polynucleotide kinase (Takara Shuzo). Poly(A)$^+$ RNA samples were prepared from Hut78, Jurkat and BALL-1, all stimulated with PHA+PMA for 24 h. Each RNA sample (3 μg) was annealed with the labeled primer and incubated with Superscript II reverse transcriptase (Gibco-BRL). The extension products were electrophoresed on a 6% denaturing polyacrylamide gel along with sequencing products generated using the same primer.

RNase protection was also carried out essentially as described previously [7]. In brief, a 228 bp fragment of the *SCM-1α* gene including the 154 bp upstream of the translation initiation site was amplified by PCR using primers +5′-TTAAGAAAAATAAAAGC-3′ and −5′-CCTTCCACAATGTATGCAGTGAGAGAGCAGATGCC-3′, and subcloned into the pCRII vector (Invitrogen, San Diego, CA). The construct was then linearized at the 5′ end of the insert by *Xho*I, and transcribed in vitro by the T7 promoter to generate ^{32}P-labeled antisense RNA. Poly(A)$^+$ samples were prepared from Hut78, Jurkat and BALL-1, all stimulated for 24 h with PHA + PMA. Each RNA sample (3 μg) was mixed with the probe (5×10^5 cpm) in 20 μl of hybridization buffer (80% formamide, 40 mM PIPES, pH 6.5, 400 mM NaCl and 1 mM EDTA), denatured at 90°C for 5 min, and incubated at 50°C for 18 h. Following hybridization, samples were incubated with RNase A and RNase T1 at 37°C for 40 min. RNA was fractionated on a 6% polyacrylamide gel containing 8 M urea and detected by autoradiography.

3. Results and discussion

3.1. Cloning and genomic organization of two SCM-1 genes

We screened a human whole blood genomic library with the SCM-1 cDNA and isolated seven independent positive clones. Two clones contained a 5.3-kb *Eco*RI fragment and five clones contained a 3.8-kb *Eco*RI fragment. The entire nucleotide sequences of a clone hg40 containing the 5.3-kb *Eco*RI fragment and a clone hg44 containing the 3.8-kb *Eco*RI fragment were determined. Each clone was found to contain a complete SCM-1 gene. The SCM-1 gene in the 5.3-kb *Eco*RI fragment which corresponded to the reported SCM-1 cDNA [3] was named the *SCM-1α* gene. The other gene, present in the 3.8-kb *Eco*RI fragment, was named the *SCM-1β* gene. We consider the *SCM-1β* gene also functional because its coding regions have no base changes generating stop codons or frame shifts, and the coding regions match exactly to a minor species of lymphotactin cDNA that had been presumed to encode an allelic form of lymphotactin [5].

The organization of the two SCM-1 genes is depicted schematically in Fig. 1, and the entire nucleotide sequences are presented in Fig. 2. Both genes are composed of three exons interrupted by two introns located at the same positions. The sequences of the exon-intron junctions conform to the consensus sequences of the eukaryotic splice junctions [10]. The two genes are highly homologous except for the 1.5-kb sequence present only in the 1st intron of the *SCM-1α* gene (indicated by lower-case letters in Fig. 2A). Computer analysis revealed that the 1st intron of the *SCM-1α* gene contains an insert of 889 bp showing 83% homology to the coding sequence of the *L7a/Surf-3* gene which encodes the ribosomal large subunit L7a (Figs. 1 and 2A) [11,12]. This L7a sequence, being oriented in the opposite direction, is obviously a pseudogene with multiple in frame stop codons. It is known that multiple copies of the L7a pseudogenes exist in various mammalian genomes [11,12]. In the *SCM-1β* gene, a 1.5-kb frag-

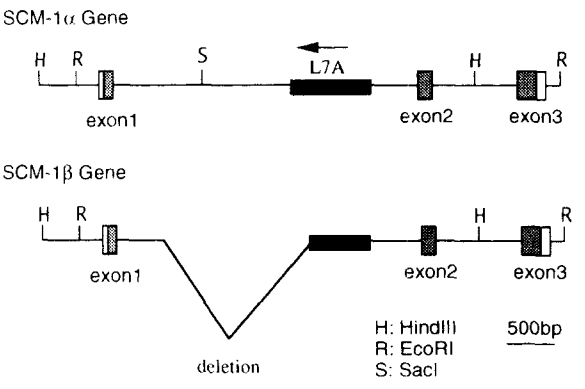
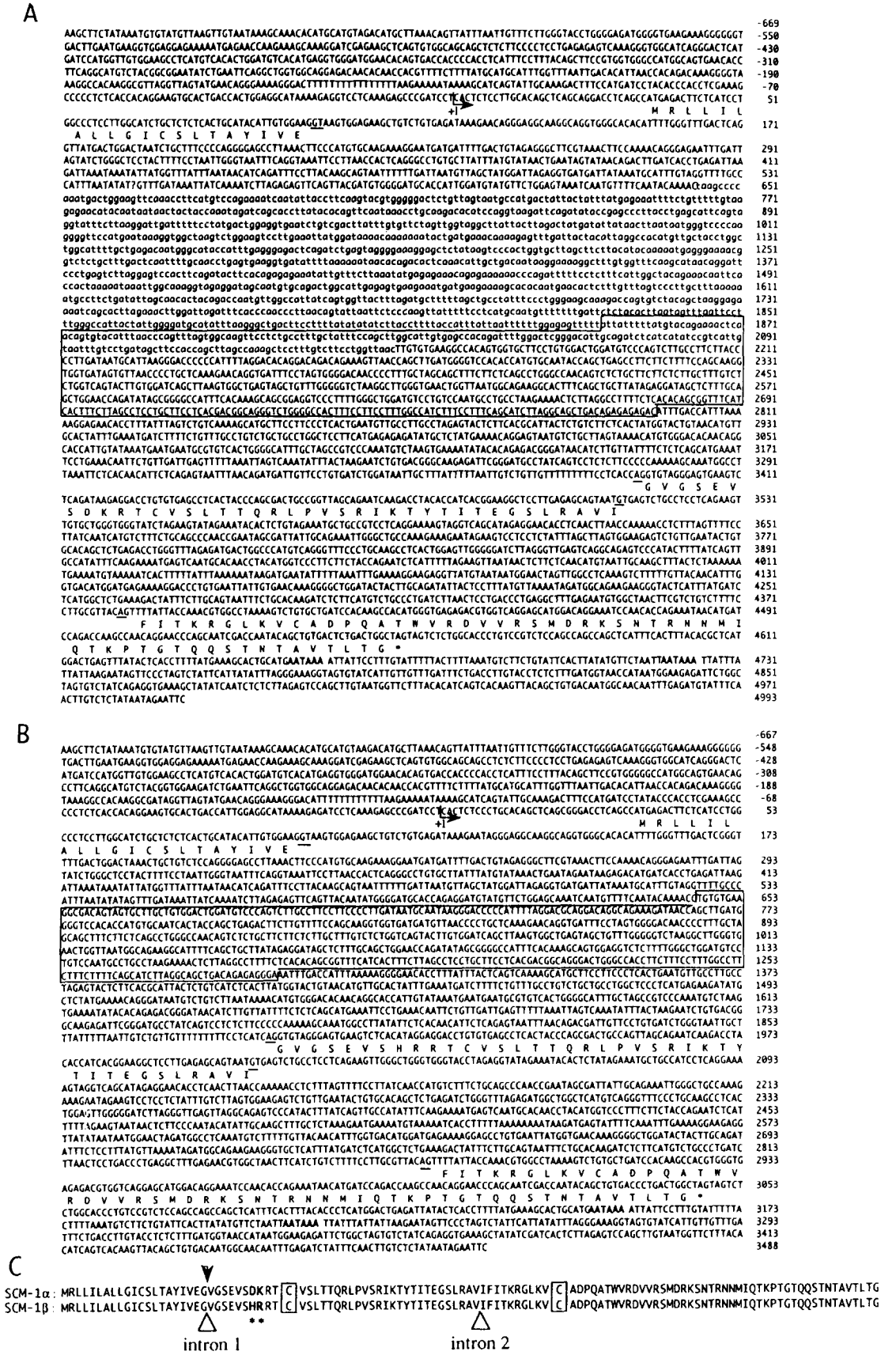


Fig. 1. Schematic representation of the organization of the two SCM-1 genes. Shaded boxes represent coding regions, open boxes denote noncoding regions, and filled boxes correspond to the L7a pseudogene sequence. The arrow indicates the orientation of the L7a pseudogene.



ment including about 1/4 of the L7a pseudogene sequence is deleted (Figs. 1 and 2B). The 0.7-kb 5′ flanking sequences are 97.5% homologous between the two genes. Similarly, the 0.4-kb 3′ flanking sequences are 99% homologous between the

Fig. 2. Nucleotide sequences of the *SCM-1α* (A) and *SCM-1β* (B) genes. A major transcriptional start site at 34 bp upstream of the initiation codon is marked by an arrow and numbered as +1. The splice donor and acceptor sites are underlined. The putative polyadenylation signals are indicated by bold letters. The L7a pseudogene sequences are boxed. The 1.5-kb sequence in the first intron of the *SCM-1α* gene that is deleted in the *SCM-1β* gene is indicated by lower-case letters. The nucleotide sequence data have been deposited in the DDBJ/EMBL/GenBank with accession numbers of D63790 for the *SCM-1α* gene and D63789 for the *SCM-1β* gene. (C) Alignment of amino acid sequences of SCM-1α and SCM-1β. Amino acid differences between SCM-1α and SCM-1β are indicated by asterisks. The two conserved cysteine residues are boxed. The cleavage site of leader sequences and positions of introns are indicated by an arrow and triangles, respectively.
←

two genes with two typical polyadenylation signals (AAUAAA) and one mRNA-destabilization signal (UUAUUUAUU) [13,14] immediately after the second polyadenylation signal.

The positions of the exon/intron boundaries relative to the protein sequences (Fig. 2C) are identical to the reported CC chemokine genes; the first exon encodes most of the leader peptide, the first intron splits a codon between the first and second bases, and the second intron splits a codon for a hydrophobic amino acid between the second and third codons [15]. It is thus likely that the original SCM-1 gene arose from the CC chemokine subfamily. Later, the insertion of an *L7a* pseudogene took place in the 1st intron of the original *SCM-1* gene. This gene was then duplicated. Subsequently, the 1.5-kb region in the 1st intron containing a part of the *L7a* pseudogene was deleted from one gene, resulting in *SCM-1β*.

The coding regions of the *SCM-1α* and *SCM-1β* genes show 97% identity with only five nucleotide changes (Fig. 2A,B). The amino acid sequences deduced from the coding sequences of the *SCM-1α* and *SCM-1β* genes are identical to each other except for two adjacent amino acids at positions 28 and 29 (7 and 8 in the mature proteins): Asp-Lys in SCM-1α and His-Arg in SCM-1β (Fig. 2C). The cleavage sites for the leader peptides of SCM-1α and SCM-1β are predicted to be between glycine and valine at amino acid positions 21 and 22 (Fig. 2C) from their homologies to the processing sites of other chemokine proteins, from the rule of Von Heijne [16], and from the N-terminal amino acid sequencing of purified SCM-1α secreted from Tn5B-4 insect cells infected with a recombinant baculovirus (unpublished results). The calculated molecular weights and p*I* values of the mature protein are 10271 and 11.10 for SCM-1α, and 10321 and 11.69 for SCM-1β.

3.2. *Southern blot analysis of human genomic DNA with the SCM-1 cDNA*

Genomic DNA samples derived from various human cell lines were digested with *Eco*RI, electrophoresed, blotted onto filters, and hybridized with the full-length SCM-1 cDNA. As shown in Fig. 3A, two *Eco*RI bands with sizes of 5.3 and 3.8 kb were hybridized with similar intensities in all the samples. The same two *Eco*RI bands were also seen with DNA samples from individuals with different racial backgrounds (Fig. 3B). These results indicate that the two highly homologous SCM-1 sequences are indeed derived from independent genes and not due to an allelic polymorphism.

3.3. *Chromosomal localization of the SCM-1α and SCM-1β genes*

Our previous segregation analysis of human-rodent somatic cell hybrids for the *SCM-1* gene using PCR demonstrated that the gene was located on human chromosome 1 [3]. By a similar hybrid segregation analysis, the same conclusion was reported for lymphotactin [5]. Another study also describing the same cDNA carried out FISH analysis and determined the locus of its gene as 1q23 [17]. This study, however, provided little information about the employed genomic probe. To reexamine the locations of the two genes, we carried out FISH analysis using the 5.3- and 3.8-kb *Eco*RI genomic fragments as probes. Using either probes, symmetrical double signals were detected only on chromosome 1q23 and no other hybri-

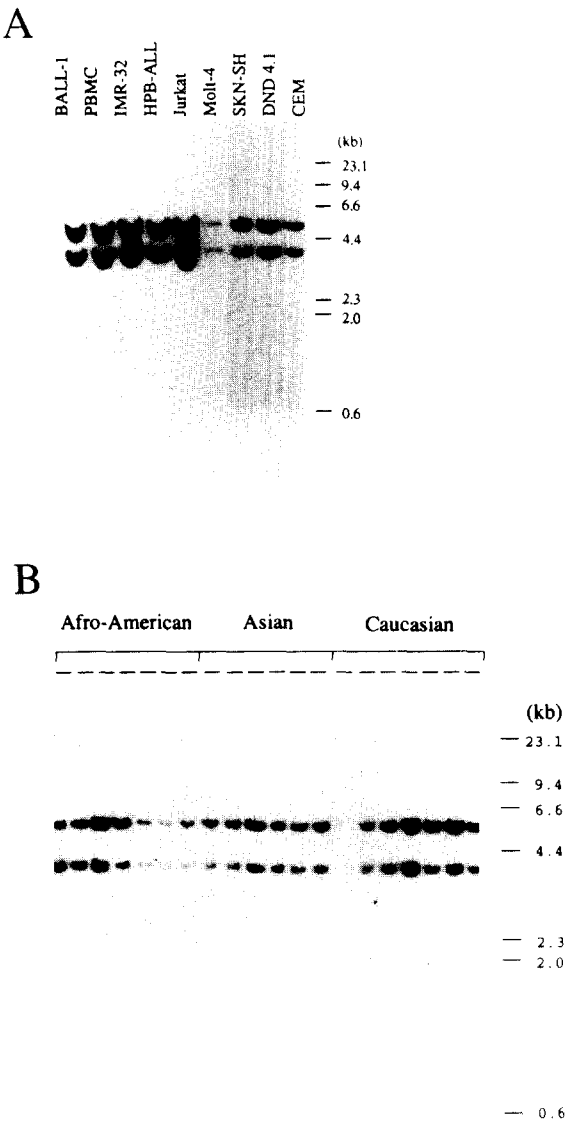


Fig. 3. Southern blot analysis of human genomic DNA demonstrating the two SCM-1 genes. (A) DNA samples (~20 μg each) isolated from various human cell lines and normal PBMC or (B) DNA samples (~8 μg each) from 19 individuals representing three different races were digested with *Eco*RI, gel-electrophoresed, blotted, and hybridized with the full-length SCM-1 cDNA. After washing, bands were visualized by autoradiography. The size markers (kb) on the right were determined using a *Hin*dIII digest of λ DNA.

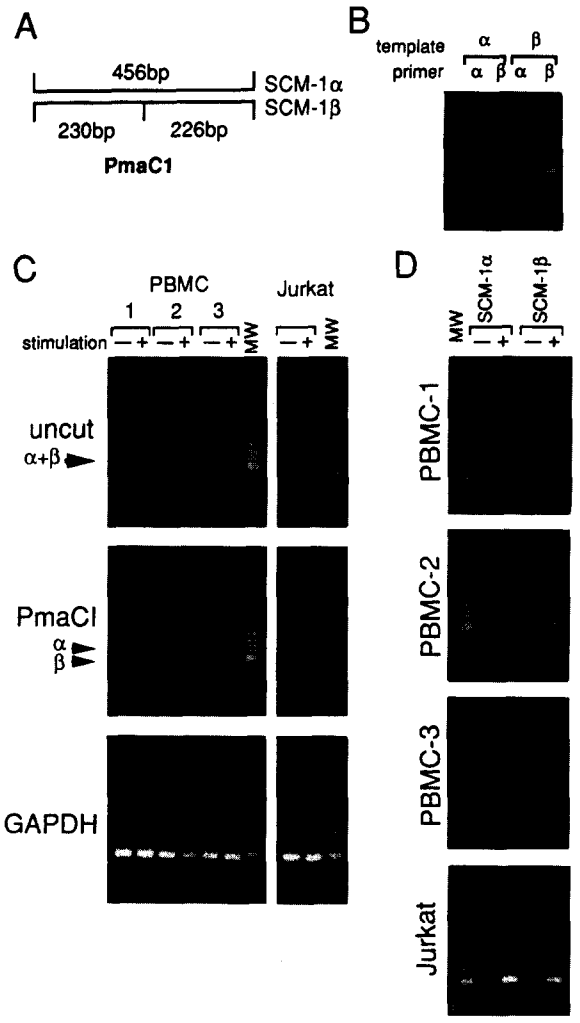


Fig. 4. RT-PCR analysis for expression of the *SCM-1α* and *SCM-1β* mRNA. (A) Predicted length of restriction fragments digested with *Pma*CI after PCR amplification with common primers for both the *SCM-1α* and *SCM-1β* sequences (method 1). Because of their almost identical sizes, the 230- and 226-bp *Pma*CI fragments were hardly separated on the gel. (B) Specific amplification of the *SCM-1α* and *SCM-1β* genomic sequences by a sense primer specific for *SCM-1α* or *SCM-1β* and a common antisense primer (method 2). (C) Method 1. Poly(A)$^+$ RNA samples were prepared from PBMC (three individuals) stimulated without (−) or with (+) PHA + PMA for 24 h and from Jurkat T cell line stimulated without (−) or with (+) PHA for 24 h. RNA was reverse-transcribed using oligo(dT) and resultant cDNA was amplified with common primers for *SCM-1α* and *SCM-1β*. Amplification products without or with *Pma*CI digestion were fractionated on a 2% GTG agarose gel. Fragments specific for SCM-1α and SCM-1β are indicated by arrows. Samples were also amplified with primers for GAPDH as internal control. Size markers are ϕX174 DNA digested with *Hinc*II. (D) Method 2. The same RNA templates were amplified by RT-PCR using specific primers for *SCM-1α* or *SCM-1β*. Size markers are ϕX174 DNA digested with *Hinc*II.

dizing signals were detected (data not shown). We, therefore, conclude that the two genes are closely located in the q23 region of human chromosome 1.

3.4. Detection of the SCM-1α and SCM-1β transcripts

Previous studies using Northern blot analysis showed that the SCM-1/lymphotactin gene was strongly induced upon mitogenic stimulations in PBL [3,5,17]. However, Northern blot analysis was incapable of distinguishing the *SCM-1α* and *SCM-1β* mRNAs because of their high sequence homology. To determine separately the expression of the *SCM-1α* and *SCM-1β* genes, we carried out RT-PCR analysis [9]. The first one employed common primers for both *SCM-1α* and *SCM-1β* mRNA. The length of the amplified DNA was 456 bp for both mRNAs, but the products from each gene could be distinguished by *Pma*CI digestion; only the *SCM-1β* gene contains one *Pma*CI site and thus gives rise to 230- and 226-bp fragments (Fig. 4A). The second one took advantage of the two base differences in the first exon of these two genes; the 5′ primers were specific for either *SCM-1α* or *SCM-1β* mRNA and the 3′primer was common for both mRNAs. It was confirmed that these primers were capable of amplifying the respective SCM-1 genes without cross-amplification (Fig. 4B). The primers for GAPDH were used for internal control. PBMC from three donors as well as Jurkat cells were cultured without or with mitogenic stimulation for 5 h and poly(A)$^+$ RNA samples were prepared. Using these RNA samples as templates, cDNAs were synthesized with oligo(dT) and amplified by PCR. We obtained quite similar results by using the two RT-PCR methods.

By the first method (Fig. 4C), we observed 456-bp amplification products in PBMC and Jurkat that were induced by mitogenic stimulations. By digestion with *Pma*CI, products from SCM-1α and SCM-1β mRNA were separately observed. Both genes were found to be induced upon stimulation. Low levels of expression in unstimulated PBMC samples appeared to be mostly from the *SCM-1β* gene. In Jurkat cells, both genes showed low levels of basal expression. By the second method (Fig. 4D), the specific primers for SCM-1α and those for SCM-1β amplified respective bands that were induced in PBMC and Jurkat upon mitogenic stimulations. Low levels of expression were seen especially for the *SCM-1β* gene in unstimulated PBMC samples. Both genes were also expressed at low levels in unstimulated Jurkat. RT-PCR for GAPDH confirmed similar amounts of mRNA in all the samples. Collectively, these results indicated that both the *SCM-1α* and *SCM-1β* genes are inducible by mitogenic stimulation. Furthermore, low levels of basal expression are often seen especially from the *SCM-1β* gene.

3.5. Mapping the transcription initiation sites

In order to determine the transcriptional initiation sites of the *SCM-1* genes, we first examined various human leukemic cell lines [6] for expression of the *SCM-1* genes by Northern

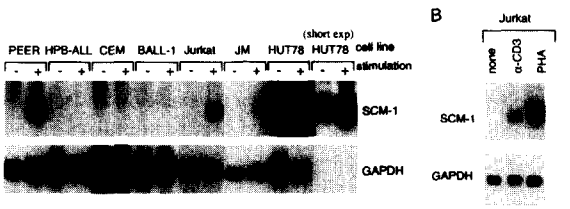


Fig. 5. Northern blot analysis of SCM-1 mRNA expressed in various human lymphoid cell lines. (A) Poly(A)$^+$ RNA samples were prepared from indicated cell lines stimulated without (−) or with (+) PHA+PMA for 24 h. (B) Poly(A)$^+$ RNA samples were prepared from Jurkat stimulated without (−) or with immobilized anti-CD3 or PHA for 24 h. RNA samples (2 μg/lane) were fractionated on a 1% agarose gel containing formaldehyde, blotted onto a filter membrane, and hybridized with ^{32}P-labeled SCM-1 cDNA. The same filters were rehybridized with a probe for GAPDH as internal control.

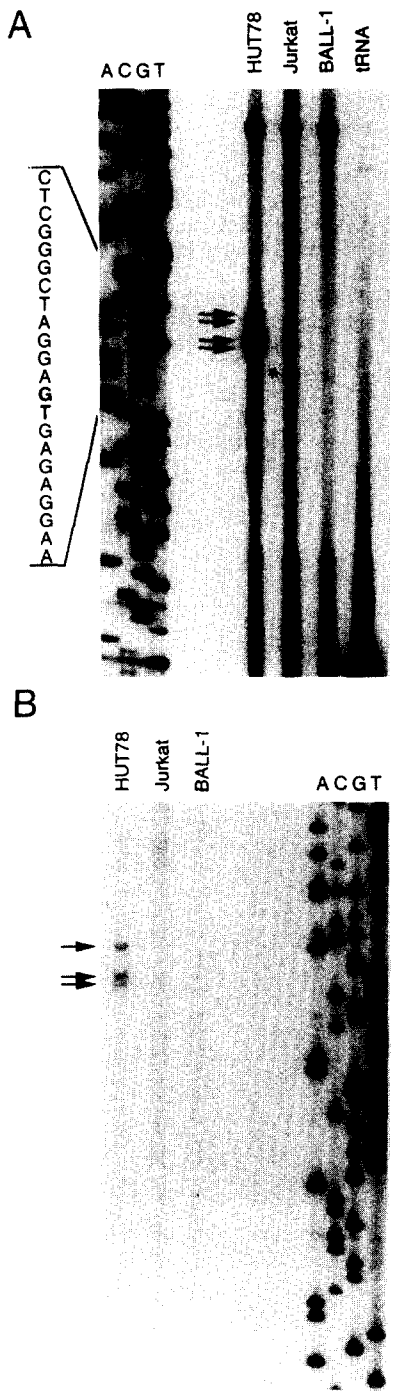


Fig. 6. Determination of the transcription initiation sites of the *SCM-1* genes. (A) Primer extension analysis. Hut78, Jurkat and BALL-1 were stimulated with PHA+PMA for 24 h and poly(A)$^+$ RNA samples were prepared. A 5′-labeled synthetic oligonucleotide complementary to nucleotides 28 to 62 downstream of the initiation codon of the *SCM-1α* and *SCM-1β* genes (Fig. 2) was hybridized to poly(A)$^+$ RNA samples (each 3 μg). After extension, extended products were resolved on a 6% polyacrylamide/urea gel along with a sequencing ladder generated by the same primer with a template of the *SCM-1α* genomic DNA that spans the first exon. Arrows indicate the sites of transcription initiation and an asterisk indicates a nonspecific band. (B) RNase protection assay. A ^{32}P-labeled RNA probe (341 nucleotides) was annealed with 3 μg of poly(A)$^+$ RNA samples (see above), digested with RNase A+RNase T1, and fractionated on a 6% polyacrylamide/urea gel along with a sequencing ladder generated by the T7 primer. Protected bands with sizes of about 95, 96 and 100 nucleotides are indicated by arrows.

blot analysis (Fig. 5A). Hut78, a CD4$^+$ T cell line derived from Sezary syndrome, demonstrated a high level of constitutive expression, which was further upregulated by PHA stimulation. No other cell lines showed such constitutive expression. PHA treatment strongly induced the *SCM-1* expression in two T-cell acute lymphoblastic leukemia (T-ALL) cell lines, Jurkat (CD4$^+$) and PEER (CD4$^-$/CD8$^-$), but not in three other T-ALL cell lines, JM (CD4$^+$/CD8$^+$), HPB-ALL (CD4$^+$/CD8$^+$) and CEM (CD4$^+$), or a B-ALL cell line, BALL-1. In Jurkat, we also found that the *SCM-1* genes were inducible by immobilized anti-CD3 which is supposed to mimic antigenic stimulation (Fig. 5B). In the case of murine lymphoid cells, CD8$^+$ T cells as well as CD4$^-$CD8$^-$ thymocytes were shown to express lymphotactin upon activation [4]. Furthermore, human CD4$^+$NK1.1$^+$ T cells were mentioned to be an important source of lymphotactin [5]. Thus, inducibility of the *SCM-1* genes in some but not all T-ALL cell lines may correlate with such specificity for T cell subsets. No human T-ALL cell lines singly positive for CD8 were, however, available for testing.

To map the transcription initiation sites of the *SCM-1α* and *SCM-1β* genes, we carried out primer extension analysis as well as RNase protection assay using poly(A)$^+$ RNA prepared from Hut78, Jurkat and BALL-1, all stimulated with PHA+PMA for 24 h. The extension primer was complementary to both the *SCM-1α* and *SCM-1β* mRNAs. As shown in Fig. 6A, several extension products were detected strongly in Hut78 and weakly in Jurkat. These extension products mapped to 33, 34, 37 and 38 bp upstream of the ATG translational start site. RNase protection assay essentially confirmed the results of primer extension analysis, even though the lengths of protected RNA fragments could not be accurately determined (Fig. 6B). The major initiation site, the cytosine at 34 bp upstream of the initiation codon (indicated as +1 in Fig. 2), matches to the cap consensus site [18], 5′-CA(C/T)(C/T)(C/T)-3′. A probable TATA box (TAAAA) but no apparent CCAAT box is found in the upstream regions.

In conclusion, we have demonstrated that there are two highly homologous genes encoding the SCM-1 proteins in the human genome. The two SCM-1 genes will be useful for elucidating the molecular mechanism of their regulated expression in CD8$^-$ T cells. The two SCM-1 proteins have two amino acid differences at positions 7 and 8 in the mature proteins. The importance of the N-terminal regions has been demonstrated for biologic activities of chemokines such as a CXC chemokine IL-8 [19,20] and a CC chemokine MCP-1 [21]. Therefore, the two SCM-1 proteins may have some differences in their biologic activities. Production of recombinant SCM-1α and SCM-1β, now in progress, will help us examine their respective functions and identify their specific receptors.

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