ESkine, a Novel β-Chemokine, Is Differentially Spliced to Produce Secretable and Nuclear Targeted Isoforms*

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‡ The abbreviations used are: ES, embryonic stem; IL11Ra, interleukin 11 receptor α; GFP, green fluorescent protein; PCR, polymerase chain reaction; PBMC, peripheral blood mononuclear cell; SEB, staphylococcal enterotoxin B.

Using the murine embryonal stem cell system, we have identified a novel protein encoding a highly divergent member of the β-chemokine family of proinflammatory mediators and have called this protein ESkine. Much of the coding sequence for ESkine overlaps with the 3′-end of a novel interleukin 11 receptor α-like sequence on murine chromosome 4. ESkine is produced as two splice variants. One of these variants encodes a classical chemokine with an associated signal peptide, while the other variant (PESKY) possesses the main body of the chemokine but has replaced the signal peptide with an alternative stretch of amino acids that allows for nuclear targeting of this isoform. This differential splicing arises as a result of alternative 5′ exon usage. These differentially spliced forms are expressed at discrete tissue loci. Thus, while ESkine is highly expressed in the placenta, PESKY is mainly expressed in the Testes and brain and weakly in the developing embryo. Studies on the proinflammatory properties of ESkine reveal it to be active in inducing polarization of CD4+ T cells but to be inactive on other hemopoietic cellular populations.

In depth molecular analyses of hemopoietic stem cells (1) have been hampered by the numerous technical difficulties associated with primary adult stem cells such as the near impossibility of homogeneously purifying the stem cell for molecular analysis. To facilitate such studies, we have attempted to adapt the murine embryonal stem (ES) cell system (2) as an in vitro model of hemopoietic stem cell generation and function. ES cells are derived from the day 3.5 murine blastocyst and are totipotent, having the full capacity to generate all tissue of a mature mouse (3, 4). These cells are the basis for all current in vitro model of hemopoietic stem cell generation and function. Most of these receptors display a marked promiscuity (15). The novel chemokine identified in the ES cell subtracted library is a member of the β-chemokine family and shows weak homology to the chemokines LARC and TECK. This novel chemokine, which we have labeled ESkine, is structurally unique among known chemokines, has a highly divergent sequence, and overlaps at its 3′-end with a murine interleukin 11 receptor α (IL11Ra) homologue on murine chromosome 4. ESkine is generated as two alternatively spliced, differentially expressed variants. One of these variants carries a classical signal peptide, which presumably allows for release from mammalian cells and which targets ESkine to the Golgi as determined by GFP fusion protein analysis. However, the alternative form, which we refer to as PESKY, lacks the signal peptide, encoding in its place a charged domain, making it unlikely that this variant is secreted via classical endocytic pathways from mammalian cells. In fact, studies using a PESKY/GFP fusion construct indicate that PESKY translocates to the nucleus, which is maintained as its major domain of expression. These two alternatively spliced variants are expressed at discrete tissue loci, with the secretable ESkine being predominantly expressed in its place a charged domain, making it unlikely that this variant is secreted via classical endocytic pathways from mammalian cells. In fact, studies using a PESKY/GFP fusion construct indicate that PESKY translocates to the nucleus, which is maintained as its major domain of expression. These two alternatively spliced variants are expressed at discrete tissue loci, with the secretable ESkine being predominantly expressed in its place a charged domain, making it unlikely that this variant is secreted via classical endocytic pathways from mammalian cells. In fact, studies using a PESKY/GFP fusion construct indicate that PESKY translocates to the nucleus, which is maintained as its major domain of expression. These two alternatively spliced variants are expressed at discrete tissue loci, with the secretable ESkine being predominantly expressed
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The full-length cDNA for ESkine and PESKY were cloned in frame into the pEGFP-N1 expression vector (CLONTECH), which places the GFP at the carboxyl terminus of the translated proteins. For transient transfections, HEK 293 cells were maintained in Dulbecco's modified Eagle's medium with 10% fetal calf serum and 5 mM glutamine. 5 × 10⁴ cells were plated onto single well chambered slides in 3 ml of culture medium and left overnight at 37 °C, 5% CO₂. The cells were transfected using Fugene 6 (Roche Molecular Biochemicals) according to the manufacturer's instructions. Briefly, 6 µl of Fugene was added dropwise on to 94 µl of serum-free media and left for 5 min at room temperature. This Fugene mix was then added to 1.5 µg of DNA (for transfection) and allowed to stand for 15 min. This was then added to the cells in the chambered slides. Transfection was allowed to proceed for 24 h.

After 24 h, the cells were washed in PBS twice and then fixed in 3% paraformaldehyde for 30 min. They were washed again in PBS and treated with 100 µg/ml RNase A for 30 min at 37 °C. They were then mounted in Vectastain containing propidium iodoside (1.5 µg/ml) (Vector Laboratories). Preparations were examined on a Bio-Rad MRC 600 confocal attached to a Nikon Diaphot microscope. Images were taken with × 80 oil immersion lens.

Cell Polarization Assay—This assay measures the change from a spherical shape to the shape characterized by head-tail polarity, typical of locomotive cells (18–20). Heparinized venous blood was withdrawn gently from the forearm of donors. PBMCs were isolated using density gradient separation (Lymphoprep, Nycomed, Birmingham, UK) and washed in RPMI 1640 (ICN, Flow, High Wycombe, UK). Cells were used either directly or cultured overnight (3–4 × 10⁵ cells/ml) in a round bottom polystyrene tube, 17 × 100 mm) at 37 °C and 5% CO₂ in medium containing staphylococcal enterotoxin B (SEB, 1 µg/ml; Sigma; Ref. 21). SEB was made up of BSA supplemented with fetal calf serum (10 mg/ml), penicillin (100 IU/ml), streptomycin (100 mg/ml), and L-glutamine (2 mM) (all from Life Technologies). For the polarization assay, freshly isolated or overnight cultured PBMCs (2 × 10⁵ cells/0.2 ml) in conical base plastic tubes (110 × 16 mm) were exposed for 30 min at 37 °C to graded concentrations of ESkine, interleukin 8, or medium alone. The cells were then fixed by adding an equal volume of 2.5% formaldehyde (Sigma), and the proportions of total PMBC expressing the functional tail polarization typical of locomotive cells were determined. The proportion of cells scored as either spherical (nonmotile) or polarized (motile) was counted directly using a × 40 phase-contrast objective. 250–300 cells were counted blind, and polarized cells were expressed as a percentage of viable cells. For phenotyping, fixed cells were stained with fluorescein isothiocyanate-conjugated anti-CD3, -CD4, -CD8, -CD19, -CD14, or -CD45 (Sigma) and scored under a fluorescent phase contrast microscope. Total PMBC expressing each of the phenotypic markers were analyzed with a FACScan flow cytometer (Becton Dickinson, Mountain View, CA).

Collagen Gel Invasion Assay—Rat tail collagen (type I) was prepared in solution from freshly obtained rat tail tendons by established methods (22, 23). Gels were formed by bringing soluble collagen (1.5 mg/ml) in dilute acetic acid solution back to physiologic pH and osmolarity, and ESkine (to 1 ng/ml) or human serum albumin in culture medium were added prior to gelatinization. PBMCs were prepared and activated with SEB as described above. They were washed and overlaid on the gel in 24-well dishes and incubated at 37 °C for 20 h to allow the cells to invade the gel. The proportion of invading cells was determined by scoring the number of cells remaining on top of the gel and the number of cells that had penetrated the gel, counting a minimum of 200 cells with an inverted microscope. The proportion of locomotor cells was calculated from the ratio between the numbers of invasive and noninvasive cells.

RESULTS

Clone JB438 Encodes a Novel β-Chemokine—Initial data base searching with one of the clones (JB438) from the ES cell subtracted library identified numerous homologous ESTs in the murine data base. These ESTs have high homology (82%) to the extreme 3'-end of exon 13 in the untranslated region of the murine interleukin 11 receptor α-chain (24–26) and it was initially assumed that JB438 represented a novel, highly conserved, member of this receptor family. Surprisingly, closer analysis of JB438 and the related ESTs indicated that while the homology with the IL11Rα gene was identifiable in one reading orientation, conceptual translation of the cDNAs in the reverse orientation indicated that JB438 and the homologous

EXPERIMENTAL PROCEDURES

Isolation of the Full-length cDNA for ESkine and PESKY—The following oligonucleotides were synthesized from the EST sequences for the isolation of full-length clones of both ESkine and PESKY: 5'-ESkine, 5'-CTCTAGGCTGATGCAGCAGT-3', 5'-PESKY, 5'-GGAAAGACCTGAGAATTG-3', 5'-ESkine and 5'-TTGGAGATTGCTCTA-3'.

Total RNA for the PCR was extracted using Trizol (Life Technologies, Inc.) according to the supplier's instructions. Day 12.5 murine placental RNA was used to isolate the ESkine cDNA, whereas murine testes RNA was used to isolate the PESKY cDNA. The RNA was DNase I-treated as described previously (16, 17) to remove genomic contamination, followed by phenol/chloroform extraction and ethanol precipitation. 1 µg of RNA was used for each reverse transcriptase-PCR reaction. The reverse transcriptase-PCR was performed using the RNA PCR core kit (Perkin-Elmer). PCR was carried out using Pfu polymerase (Stratagene) and proceeded for 30 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min. The PCR products were cloned into pCRscript (Stratagene, La Jolla, CA) and sequenced in both directions using the ABI 373A sequencer (Applied Biosystems).

Generation of RNA for Expression Analysis—All RNA samples were generated using TRIZOL (Life Technologies Inc.). For tissue blots, tissues were removed from 6-week-old C3H mice and stored in liquid nitrogen prior to RNA extraction. For embryo and placental RNA generation, timed matings were initiated, and pregnant mice were sacrificed at the required time points. Placenta and embryos were separated, and RNA was generated from both at each time point.

Generation of Radiolabeled Probes—For Northern blotting, the cDNA probes were labeled with [α-32P]dCTP (Amerham Pharmacia Biotech) using the Ready-to-go kit (Amerham Pharmacia Biotech), and unincorporated nucleotides were removed using NICK columns (Amerham Pharmacia Biotech). Probes were denatured at 100 °C for 3 min prior to hybridization. For specific detection of ESkine or PESKY sequences, the alternative 5' exons were subcloned into pCRscript and subsequently radiolabeled as cDNA fragments using the Ready-to-go kit.

mRNA Expression Analysis—For Northern blot analysis, 15 µg of total RNA, isolated using Trizol (Life Technologies) was fractionated on a 1.2% denaturing gel and subsequently blotted by capillary action onto a Hybond-N membrane (Amerham Pharmacia Biotech, Little Chatfont, UK). Blotted membranes were UV-cross-linked using a Stratalinker. Prehybridization and hybridization were both carried out in Express-Hyb (CLONTECH, Palo Alto, CA), and the blots were washed, following hybridization, as recommended by the suppliers. Membranes were exposed to Kodak X-Omat x-ray film and stripped in 0.1% SDS at 100 °C for 15 min.

Genomic Localization—Genomic localization of the murine ESkine sequence was carried out using a mouse/hamster somatic cell hybrid panel supplied by the UK HGMP resource center. This approach involves attempts to detect the murine gene in a range of murine/hamster somatic cell hybrids using PCR. For the PCR, the following primers were used: 5' primer, 5'-CTCTAGGCTGATGCAGCAGT-3'; 3' primer, 5'-TTGGAGATTGCTCTAATG-3'.

PCR was performed using 1.25 mM MgCl₂, 0.2 mM of each dNTP from the PCR core kit (Perkin-Elmer), and 0.15 µM of the 5' and 3' primers. Reactions were incubated for 30 cycles at 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min. Products from the PCRs were visualized on a 1.5% agarose gel.

Genomic Sequencing—The genomic fragment encoding ESkine and PESKY was generated by PCR. This approach assumed the PESKY cDNA sequence to contain the most 5' sequence stretches within the genomic locus, and thus the 5' genomic primer was designed using the 5' noncoding sequences of PESKY. Since both PESKY and ESkine have identical 3' sequences, a common 3' primer was generated based on the 3' noncoding sequences at this position. These primers were used in a PCR utilizing Pfu polymerase and genomic DNA from C3H mice. The generated fragment (approximately 2 kilobase pairs) was sequenced in both orientations, and a representative genomic map of the ESkine/ PESKY locus was created.

Generation and Analysis of ESkine and PESKY GFP Fusion Constructs—For analysis of the subcellular fates of ESkine and PESKY, the full-length coding sequences were cloned in frame into the pEGFP-N1 vector (CLONTECH), which places the GFP at the carboxyl terminus of the translated proteins. For transient transfections, HEK 293 cells were maintained in Dulbecco's modified Eagle's medium with 10% fetal calf serum and 5 mM glutamine. 5 × 10⁴ cells were plated onto single well chambered slides in 3 ml of culture medium and left overnight at 37 °C, 5% CO₂. The cells were transfected using Fugene 6.
ESTs encode a novel member of the β-chemokine family. We have labeled this chemokine ESkine.

The murine IL11Ra family are known to reside on chromosome 4 in the mouse (27), and using mouse/hamster cell hybrids we have demonstrated specific detection of ESkine on chromosome 4. Thus, ESkine and the IL11Ra homologue lie within the same locus in the murine genome and appear to be transcribed in different directions along chromosome 4, with ESkine overlapping with the extreme 3′-end of the IL11Ra homologue (Fig. 1). The overlap with the homologous regions of the published murine IL11Ra gene encompasses 149 bases of the 3′-untranslated region of the receptor gene. The complementary 149 bases of ESkine include 126 bases of sequence encoding the region of the mature protein from CVH ... to ... QQQN (see Fig. 2a). Thus, a substantial portion of the third ESkine coding sequence overlaps with the 3′-untranslated region of the putative novel receptor.

**ESTs AA799176 and AA271042 Encode Variants of ESkine**—Survey of the ESTs detected by JB438 indicate that EST accession number AA799176 encodes the open reading frame of ESkine, and we have confirmed this sequence using *Pfu* PCR (Fig. 2a). The predicted protein has a 24-amino acid leader sequence with cleavage being predicted between alanine 24 and leucine 25. The predicted mature ESkine is 95 amino acids in length, is highly basic with a net charge of +8, and possesses a putative N-linked glycosylation site beyond the first cysteine residue in the mature protein. The CC motif confirms ESkine as a β-chemokine and data base searching reveals weak homologies (33–36%) to the murine chromosome 4. Thus, ESkine and the IL11Ra homologue overlap at their 3′-ends, bp base pair.

**Fig. 1. Diagrammatic representation of the ESkine genomic locus.** ESkine has been mapped to murine chromosome 4 using a panel of mouse/hamster somatic cell hybrids. Data base searching reveals an overlap with a close homologue of the murine IL11Ra chain, suggesting that ESkine and this novel receptor homologue overlap at their 3′-ends. bp base pair.

Intriguingly, JB438 also detects other ESTs within the murine data bases, and one of these (AA271042) encodes the full sequence of a variant of ESkine (Fig. 2b). This variant encodes the full-length of the mature protein but has lost the signal peptide, replacing it with an alternative stretch of 32 amino acids. This novel amino-terminal stretch has not been seen in any other chemokines, has no homologies in any data bases, and is not predicted to act as a signal peptide. Furthermore, no other subcellular targeting signatures were detected in this alternative amino-terminal sequence. We have labeled this alternative, presumably nonsecreted (see below), isoform of ESkine PESKY.

**Analysis of nonmurine ESTs reveals the existence of a close rat homologue.** (GenBank™ accession number AI058901), which, as shown, is 87% identical and 88% similar to the mature murine protein (Fig. 2c). Sequences 5′ to the sequences shown in Fig. 2c indicate that this EST encodes the rat homologue of PESKY, and thus far the direct rat ESkine homologue has not been reported. Like its murine counterpart, the rat...
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Homologue is highly basic with an overall charge of +11. Additionally, the rat protein has maintained the two potential CD26 cleavage sites and the putative N-linked glycosylation site. In addition to this rat orthologue, conceptual translation of the reverse orientation of the 3′-untranslated region of the human IL11Ra genomic sequence (accession number HSU32323) reveals the presence of an ESkine homologue at this position also. The sequence of the presumed protein encoded by this genomic sequence is shown in Fig. 2c and, as can be seen, displays 62% identity and 73% similarity with murine ESkine over the deduced sequence. Intriguingly, the putative glycosylation site conserved in murine and rat ESkine is altered from NRS to NPS in the human protein. The presence of a proline residue in this motif makes it unlikely that it will be used as a glycosylation site in the human protein (33). Insufficient N-terminal sequence information is available to confirm the maintenance of the double CD26 cleavage site in this protein.

ESkine and PESKY Are Produced by Alternative Splicing—The presence of two alternative isoforms of murine ESkine suggests differential splicing, and to attempt to understand this process we have sequenced approximately 2 kilobase pairs of genomic sequence incorporating the ESkine/PESKY gene. As shown in Fig. 3, the exon-intron structure predicts the ESkine-specific 5′ exon (exon 1a) to be the most 5′ within the genomic locus. This is then followed by the ESkine alternative exon (exon 1b) and subsequently by the common exons encoding the mature chemokine. ESkine/PESKY is therefore unprecedented, since this is the first example of differential splicing of this nature and of the inclusion of PESKY-like amino-terminal sequences within the chemokine family.

ESkine and PESKY Are Differentially Expressed—Preliminary analysis of the expression of ESkine and PESKY using the JB438 sequence, which encodes the third and fourth exons, common to both ESkine and PESKY, as a probe on murine tissue dot blots, indicated expression of ESkine/PESKY in murine embryos as early as day 7.5 and additionally in testes and weakly at other body sites (Fig. 4a). The small number of cells associated with a day 7.5 murine embryo prompted us to look more closely at the site of expression here, and we have now demonstrated, again using the common cDNA portion as a probe, that the expression is predominantly in the placental tissue and is seen only weakly in the embryo (data not shown).

It is of interest to note that murine IL11Ra is also expressed in the placenta (34); however, we have ensured specific detection of ESkine by using probes capable of discriminating between ESkine and the murine IL11Ra homologue on blots. Repeated attempts to RACE the full-length ESkine/PESKY cDNAs from the placenta and the testes revealed that consistently, ESkine was detected in the placenta but not the testes, while PESKY was detected in the testes but not the placenta. This suggested differential expression of these two isoforms and indicates that the expression detected in the placenta using ESkine/PESKY common sequences is a consequence of ESkine expression at this site. We have now confirmed this, and analysis of expression in the embryo and the placenta using probes specific for the alternative 5′ exons of PESKY and ESkine revealed ESkine to be undetectable in the embryo but to be expressed at high levels in the placenta (Fig. 4b). Curiously, analysis of PESKY expression indicates absence in the placenta but its expression, albeit weak, in the embryo (Fig. 4b). Further tissue analysis using Northern blotting has failed to detect expression of ESkine-specific sequences in other tissues (data not shown). However, PESKY-specific sequences are readily detectable in the brain and testes and are weakly expressed in kidney and liver (Fig. 4c). It thus appears that ESkine and PESKY are produced through alternative splicing mechanisms and that these splice variants are predominantly expressed at distinct tissue loci. More sensitive techniques of PCR have revealed low level expression of PESKY in heart and muscle tissue, and, while PCR has not revealed additional sites for ESkine expression, numerous ESkine like ESTs have been reported as being derived from epidermal tissues; thus, the skin is a likely source for ESkine expression.

ESkine and PESKY Are Targeted to Discrete Subcellular Loci—To attempt to examine the impact of the alternative first exon usage on ESkine/PESKY subcellular targeting, we have generated fusion cDNAs in which the coding sequence for the GFP is positioned in frame with the C terminus of ESkine and PESKY. Transfection of these fusion constructs into HEK cells revealed discrete subcellular destinations for the ESkine/GFP and PESKY/GFP protein products. As shown in Fig. 5a, ESkine targets the GFP protein to a perinuclear location that has been revealed as the Golgi apparatus by co-staining with the Golgi-specific BODIPY-TR stain (data not shown). Additionally, weak fluorescence is detected throughout the cytoplasm concomitant with the processing of this secreted chemokine through the Golgi and endoplasmic reticulum. In contrast, PESKY is seen to target the fluorescence specifically to the nucleus (Fig. 5b) as shown by both phase contrast microscopy and co-localization with propidium iodide staining. Little PESKY-directed fluorescence is detected in other subcellular positions (Fig. 5b). A control GFP-expressing construct failed to target the GFP to any specific loci, and fluorescence is seen throughout the control transfected cells (Fig. 5c). Identical results have been obtained using both COS and CHO cell transfectants, confirming that these subcellular targeting differences are not exclusive to HEK cells (data not shown).

Thus, the differential splicing outlined in Fig. 3 yields two variants of the same chemokine that are differentially expressed and destined for discrete subcellular fates. The targeting of PESKY to the nucleus is the first example of nuclear targeting of a member of the chemokine family, although, as discussed below, such nuclear accumulation has been reported for a number of other growth factors such as the members of the fibroblast growth factor family.

Biological Activity of ESkine—We have encountered significant technical difficulties in the purification of recombinant ESkine from mammalian or baculovirus expression sources. For this reason, to facilitate investigations into the biological properties of ESkine, we have chemically synthesized the mature protein using previously described techniques (35). Being a member of the β-chemokine family, the predicted roles for ESkine lie in the regulation of inflammatory or immune cell function (however, see below for a discussion of this point).
Such effects on these cell populations typically lead to cell polarization, which can be quantified as outlined under “Experimental Procedures.” We have therefore examined the biological activity of ESkine in terms of its effects on polarization of resting and SEB-activated (36) human PBMCs. While ESkine had no detectable effects on polarization of resting PBMCs, SEB-activated PBMCs showed a classical “bell-shaped” dose response to activation by ESkine. Maximal polarization was seen at 1 ng/ml ESkine, at which concentration, following removal of background polarization controls, approximately 20% of PBMCs were polarized (Fig. 6). This polarizing activity is comparable with (and perhaps even slightly higher in potency than) that of interleukin 8, another well characterized chemokine (Fig. 6 and Ref. 37) and additionally is very similar in potency to MIP-1α, which we have previously shown to be maximally active at 1 ng/ml in the polarization assays (16). To investigate the phenotype of the subset of PBMCs polarized by ESkine, we have carried out an analysis using a range of lineage-specific surface markers. As can be seen from Table I, only CD3⁺ and CD4⁺ cells are polarized by ESkine. No effect on the polarization of CD8⁺ cells was observed. Additionally, no effect of ESkine was seen on the CD19⁺ B cell population or on the CD14⁺ population of monocytes.

These polarization results therefore suggest that the effects of ESkine are detectable on activated but not resting PBMCs and that the majority of cells involved in this response are CD4⁺ T cells. The relatively restricted phenotype of the polarized cells is likely to be the reason for the limited magnitude of response of the PBMCs to ESkine in the polarization assay. Additionally, this restricted responsive population makes a systematic comparison of efficacy between ESkine and other chemokines, which frequently act on wider, or alternative, subpopulations of cells, difficult.

Although measurement of polarization is a reliable correlate of chemoattraction/locomotion, the assay does not directly measure these properties. To more formally demonstrate the chemoattractant/locomotion-inducing properties of ESkine, we carried out the collagen gel invasion assay, in which PBMCs were allowed to invade collagen gels containing ESkine or control human serum albumin. Results in Table II show that the percentage of cells migrating into the gels in response to ESkine was significantly higher than control, indicating a specific ability of ESkine to induce locomotor activity in the PBMCs that is higher than that induced by the nonspecific (human serum albumin) protein controls. These results are similar to those obtained in the polarization assays (Fig. 6) and suggest that, in addition to stimulating morphological polarization, ESkine stimulates locomotion into a connective tissue matrix. It should be pointed out that the levels of migration seen in these assays are lower than those seen in similar assays in which interleukin 8 has been used as the chemoattractant (37), and it is likely that this, again, is a reflection of the limited phenotype of the ESkine-responsive cells.
DISCUSSION

During the analysis of a subtracted library prepared from differentiating ES cells, we identified a novel cDNA that is identical to a number of ESTs in the murine database. The homologous ESTs are very similar to the extreme 3'-end of the murine interleukin 11 receptor α-chain, suggesting initially that they represented novel members of this receptor family.

Closer inspection of the ESTs revealed that reverse translation indicates that they encode a novel β-chemokine, which we have called ESkine. Both ESkine and the murine IL11Rα homologue lie on murine chromosome 4 and overlap with each other at their extreme 3'-ends. This type of genomic organization of two separate genes with 3' overlaps has been previously reported (38–41); however, it is currently without precedent in the chemokine family.

We are currently uncertain whether the novel IL11Rα-like sequences represent a previously uncharacterized member of this receptor family or simply a duplicated 3' fragment from the IL11Rα genomic locus. Arguing against this being a novel full-length IL11Rα gene are earlier studies that have indicated the absence of novel IL11Rα isoforms in the murine genome (24, 26). We are currently sequencing ESkine genomic clones and hope to be in a position soon to understand the nature of the novel IL11Rα-like sequences. Whatever the precise nature of these sequences, it is clear that there is an evolutionarily conserved relationship between ESkine/PESKY and the IL11Rα family. Indeed, at the extreme 3'-end of the published murine IL11Rα, there is a partial stretch of ESkine-like sequences; however, this is interrupted by numerous stop codons. In a similar manner, reverse translation of the extreme 3'-end of human IL11R reveals human ESkine to be encoded at the equivalent locus.

Further analysis of ESkine-related ESTs reveals two isoforms of this chemokine that are generated by alternative 5' exon splicing. One of these forms encodes a signal peptide-bearing variant (ESkine), while the other (PESKY) replaces the signal peptide with an alternative stretch of amino-terminal amino acids that is not predicted to function as a signal peptide. Data base searching has failed to reveal any subcellular targeting motifs within this alternative amino-terminal sequence. Results from transfections of HEK and CHO cells with ESkine/GFP and PESKY/GFP fusion constructs indicate that while, as expected, ESkine appears to be localized over the endoplasmic reticulum and Golgi apparatus, PESKY appears to target GFP to the nucleus. Nuclear translocation of growth factors has been reported previously (42–44), and in the case of members of the fibroblast growth factor family, this nuclear translocation appears to be essential for aspects of function (45, 46). The highly basic nature of PESKY is a likely contributor to its nuclear localization, and we are currently attempting to identify the specific nuclear targeting signal within the complete PESKY sequence. It is not yet clear whether the PESKY-
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Specific sequences are responsible for the nuclear targeting or whether they simply act to block signal peptide-directed secretion. In this event, the nuclear localization signal is likely to reside within the mature chemokine sequence common to both ESkine and PESKY. This may therefore suggest that upon internalization following receptor binding, ESkine also will be translocated to the nucleus of its target cells. Evaluation of this possibility awaits the identification of the ESkine cellular receptor. This is the first example of nuclear targeting of a member of the chemokine family, and the elucidation of the role of nuclear PESKY will be central to our understanding of the role of this “intraEMK” in the cells in which it is expressed.

Expression studies have revealed the two isoforms to be detectable in discrete tissues. Thus, while ESkine is only de-

![Figure 6](image1.png)

**Figure 6.** Dose response of human peripheral blood mononuclear cells to ESkine in polarization assays. Cells were tested directly after separation (open symbols) or cultured overnight (closed symbols) with SEB (1 μg/ml). Cells were then washed and exposed for 30 min to ESkine (squares), recombinant human interleukin 8 (circles), or medium alone. They were then washed, fixed, and scored under phase contrast microscopy for shape change (polarization). The percentage of polarized cells exposed to medium alone (4.3 ± 0.7 for freshly prepared cells, 25.7 ± 2.3% for cells cultured with SEB) has been subtracted from the figure. Data are mean of three experiments from three donors. Cells cultured with SEB and then exposed to ESkine (10 pg/ml to 10 ng/ml) were significantly different (p < 0.05) from those exposed to medium alone. Freshly isolated cells exposed to ESkine were not significantly different from those exposed to medium alone.

**Table I**

*Cell type analysis of the polarization response to ESkine*

Peripheral blood mononuclear cells were cultured for 20 h with SEB (1 μg/ml), washed, and then cultured for 30 min with ESkine (1 ng/ml) or medium alone. The cells were then washed, fixed, and stained with fluorescein isothiocyanate-conjugated antibody specific for the CD antigen indicated. The percentage of polarized cells of each phenotype was then scored under phase-contrast fluorescent microscopy. The percentage of each of the CD-positive cell types was analyzed by flow cytometry. Data are mean ± S.D. of three individual experiments from three donors. Numbers in boldface type are significantly different (p < 0.05) from the medium control.

| Cell marker | Cells positive for the marker | Marker positive cells that are in polarized morphology |
|-------------|-------------------------------|-----------------------------------------------------|
|             | %                             | Medium control ESkine (1 ng/ml)                      |
| CD3         | 64.7 ± 0.5                    | 24.7 ± 3.2                                          |
| CD4         | 40.8 ± 0.5                    | 20.7 ± 3.2                                          |
| CD8         | 19.5 ± 0.6                    | 35.0 ± 4.6                                          |
| CD9         | 7.8 ± 0.7                     | 10.0 ± 2.0                                          |
| CD14        | 8.5 ± 0.5                     | ND a                                                 |
| CD45        | 95.4 ± 1.4                    | NT a                                                 |

a ND, not detectable.

b NT, not tested.

Specific sequences are responsible for the nuclear targeting or whether they simply act to block signal peptide-directed secretion. In this event, the nuclear localization signal is likely to reside within the mature chemokine sequence common to both ESkine and PESKY. This may therefore suggest that upon internalization following receptor binding, ESkine also will be translocated to the nucleus of its target cells. Evaluation of this possibility awaits the identification of the ESkine cellular receptor. This is the first example of nuclear targeting of a member of the chemokine family, and the elucidation of the role of nuclear PESKY will be central to our understanding of the role of this “intraEMK” in the cells in which it is expressed.

Expression studies have revealed the two isoforms to be detectable in discrete tissues. Thus, while ESkine is only de-

**Note Added in Proof**—ESkine has recently been independently characterized by two other groups as ALP (Biochem. Biophys. Res. Commun. (1999) 258, 737–740) and cutaneous T cell attracting chemokine (CTACK) (A. Zlotnik, personal communication). This chemokine will also be known as CCL27 in keeping with the newly introduced chemokine nomenclature system.

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