Human Thymic Stromal Lymphopoietin Preferentially Stimulates Myeloid Cells

Pedro A. Reche, Vassili Soumelis, Daniel M. Gorman, Teresa Clifford, Man-ru Liu, Marilyn Travis, Sandra M. Zurawski, Jim Johnston, Yong-Jun Liu, Hergen Spits, Rene de Waal Malefyt, Robert A. Kastelein and J. Fernando Bazan

http://www.jimmunol.org/content/167/1/336

References This article cites 32 articles, 17 of which you can access for free at: http://www.jimmunol.org/content/167/1/336.full#ref-list-1

Subscription Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Human Thymic Stromal Lymphopoietin Preferentially Stimulates Myeloid Cells

Pedro A. Reche, Vassili Soumelis, Daniel M. Gorman, Teresa Clifford, Man-ru Liu, Marilyn Travis, Sandra M. Zurawski, Jim Johnston, Yong-Jun Liu, Hergen Spits, Rene de Waal Malefyt, Robert A. Kastelein, and J. Fernando Bazan

The sequence of a novel hematopoietic cytokine was discovered in a computational screen of genomic databases, and its homology to mouse thymic stromal lymphopoietin (TSLP) suggests that it is the human orthologue. Human TSLP is proposed to signal through a heterodimeric receptor complex that consists of a new member of the hemopoietin family termed human TSLP receptor and the IL-7R α-chain. Cells transfected with both receptor subunits proliferated in response to purified, recombinant human TSLP, with induced phosphorylation of Stat3 and Stat5. Human TSLPR and IL-7Rα are principally coexpressed on monocytes and dendritic cell populations and to a much lesser extent on various lymphoid cells. In accord, we find that human TSLP functions mainly on myeloid cells; it induces the release of T cell-attracting chemokines from monocytes and, in particular, enhances the maturation of CD11c+ dendritic cells, as evidenced by the strong induction of the costimulatory molecules CD40 and CD80 and the enhanced capacity to elicit proliferation of naïve T cells. The Journal of Immunology, 2001, 167: 336–343.

Tyrosine phosphorylation of the transcription factor Stat5, but whereas IL-7-mediated signaling occurs via activation of Janus kinases JAK1 and JAK3, TSLP is unable to activate either enzyme (3, 11), but may instead interact with JAK2 (10). This evidence points to as yet undefined biological effects of TSLP that are not shared with IL-7. Although mouse TSLP was first reported in 1994, no human homologue of TSLP has been identified.

Here we describe a new human hematopoietic cytokine and its corresponding primary receptor, respectively called hTSLP and hTSLPR. We provide evidence that the functional receptor for hTSLP consists of hTSLPR and hIL-7Rα, and activation of this complex leads to phosphorylation of both Stat5 and Stat3. Biologic characterization indicates that hTSLP acts primarily on myeloid cells. As suggested by the coexpression profile of TSLPR and IL-7Rα on monocytes and dendritic cells, we find that human TSLP can induce the release of T cell-attracting chemokines from monocytes and potently enhances the T cell stimulatory capacity of the CD11c+ subset of dendritic cells.

Materials and Methods

Cell lines

Human 293T epithelial cells were maintained in DMEM (Life Technologies, Gaithersburg, MD) supplemented with 10% FCS. The pro-B cell line Ba/F3 was maintained in RPMI 1640 (Life Technologies) supplemented with 10% FCS and 10 ng/ml mouse IL-3. QBI-293A human embryonic kidney cells used for adenovirus expression were grown in CMF-1 medium (CellWorks, San Diego, CA). BOSC23 cells were maintained in DMEM-10% FCS and guanine phosphoribosyltransferase selection reagents (Specialty Media, Lavellette, NJ). The cells were transfected to DMEM-10% FCS without guanine phosphoribosyltransferase selection reagents 2 days before transfection.

Adenovirus expression of human TSLP and purification of the recombinant protein

The mature coding region of human TSLP (residues 29–159) was fused to the signal sequence of mouse SLAM (12) and inserted into a modified version of transfer vector pQBI-AdCMV5-GFP (Quantum Biotechnologies) by PCR. Recombinant adenovirus was produced as described in Quantum applications manual 24AL98. Recombinant virus was used to infect 5 × 10⁶ cells in 11 CMF-1 with culture in a Nunc Cell Factory (Nalge Nunc, Naperville, IL) for 3 days. The culture medium was clarified.

DNAX Research Institute, Palo Alto, CA 94304

Received for publication March 28, 2001. Accepted for publication April 25, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 DNAX is supported by Schering Plough Corp. (Bloomfield, NJ).
2 Address correspondence and reprint requests to Dr. Robert Kastelein, DNAX Research Institute, 901 California Avenue, Palo Alto, CA 94304-1104. E-mail address: kastelein@dnax.org
3 Abbreviations used in this paper: TSLP, thymic stromal lymphopoietin; mIL-3, murine IL-3; mTSLP, mouse TSLP; hTSLP, human TSLP; TSLPR, TSLP receptor; Rγc, common γ-chain receptor; JAK, Janus kinase; DC, dendritic cells; TARC, thymus and activation-regulated chemokine; MDC, macrophage-derived chemokine; PARC, pulmonary and activation-regulated chemokine.
by centrifugation, dialyzed, and filtered before application to a 5-ml Q-Sepharose column. The Q-Sepharose flow-through, which contained human TSLP, was loaded onto a 5-ml HiTrap heparin (Pharmacia, Uppsala, Sweden) column at 5 ml/min. The column was washed with 50 mM Tris-HCl (pH 8.0) and 1 mM EDTA and eluted with a gradient from 0 to 2.5 M NaCl in 50 mM Tris-HCl (pH 8.0) and 1 mM EDTA. The peak fractions were concentrated, dialyzed against PBS and quantitated by SDS-PAGE and Coomassie staining using lysozyme as a standard. A similar procedure was followed to prepare mouse TSLP.

**Ba/F3 retroviral-mediated gene transfer and proliferation assays**

Human IL-7Ra cDNA and human TSLPR cDNA were cloned by PCR in the retroviral vectors pMX and its derivative pMX-puro to give pMX-hIL-7Ra and pMX-puro-TSLPR, respectively (13). The BOSC23 packaging cell line was transiently transfected with retrovirus constructs using Fugene 6 (Life Technologies) according to the manufacturer’s protocol. Retroviruses-containing supernatants were collected after 2 days. Ba/F3 cells were infected with retroviral supernatants for 48 h on petri dishes coated with 40 μg/ml recombinant fibronectin fragments (Retronection; Takara, Shiga, Japan). After 48 h purinocycin (1 μg/ml) was added to those cells infected with virus obtained from pMX-puro constructs. The efficiency of infection of Ba/F3 cells was >90% as assessed by parallel infection with the test construct pMXI-EGFP encoding the enhanced green-fluorescent protein (EGFP). Proliferation assays using Ba/F3 cells were performed as previously described (14). Cells were washed three times with RPMI medium and plated at a density of 5000 cells/well. Cells were grown with serial 3-fold dilutions of mouse IL-3, human and mouse TSLP, or human IL-7 (all starting concentrations of 225 ng/ml). After 36 h at 37°C, Alamar Blue (BioSource International, Camarillo, CA) was added to each well at a final concentration of 10% (v/v) to each well. Cells were allowed to grow for 5–8 h more, after which plates were measured with a fluorometer.

**Quantitation of mRNA expression**

cDNAs from various tissue and cellular sources were prepared as described previously (15) and used as templates for Taqman-PCR analyses. cDNAs (50 ng/reaction) were analyzed for the expression of hTSLP, hTSLPR, and hIL7Ra genes by the fluorogenic 5’-nucleic PCR assay (16), using an ABI Prism 7700 Sequence Detection System (Perkin-Elmer, Foster City, CA). Reactions were incubated for 2 min at 50°C, denatured for 10 min at 95°C, and subjected to 40 two-step amplification cycles with annealing/extension at 60°C for 1 min, followed by denaturation at 95°C for 15 s. The amplicons used for hTSLP, hTSLPR, and IL-7Ra covered bp 246–315, 263–335, and 519–596, respectively (numbering starts at the start codon), and were analyzed with 6-carboxyfluorescein (FAM)-labeled probes. Values were expressed as femtograms per 30 ng total cDNA. Primers and probes for human chemokine and chemokine receptors were obtained from Perkin-Elmer as predeveloped assay reagents. Chemokine and chemokine receptor expression was adjusted for the amount of 18S rRNA and compared with the control (calibrator) samples using the comparative ∆∆ Ct method (17). Samples were measured in duplicate. 18S rRNA levels were determined under primer-limited conditions in multiplex reactions as recommended using a Vic-labeled probe (Perkin-Elmer).

**Cell isolation and culture**

PBMC were purified from buffy coats of healthy volunteers (Stanford Blood Bank, Palo Alto, CA) by centrifugation over ficoll. Human monocytes were isolated from PBMC by negative depletion using anti-CD2 (Leu 5A), anti-CD3 (Leu 4), anti-CD8 (Leu 2a), anti-CD19 (Leu 12), anti-CD20 (Leu 19), anti-CD6 (Leu 19), and anti-CD65 (BD PharMingen, San Jose CA), anti-CD67 (IOM 67; Immunotech, Westbrook, ME), and anti-glycyophorin A (107F MN; American Type Culture Collection, Manassas, VA) mAbs and sheep anti-mouse IgG-coupled magnetic beads (Dynal, Rockford, IL). All sorted cells were cultured in RPMI 1640 containing 10% FCS at 5 × 10⁶ cells/ml in flat-bottom 96-well half-area plates or at 1 × 10⁸ cells/ml in flat-bottom 96-well plates, with or without TSLP (15 ng/ml).

**TARC ELISA**

The production of trymus and activation-regulated chemokine (TARC/CCL17 in culture supernatants was determined by chemokine-specific ELISA using MAB364 as capture reagent and BAF364 as detection reagent (R&D Systems, Minneapolis, MN). The sensitivity of the assay was 50 pg/ml.

**DC viability and flow cytometric analysis**

After 24 h of culture, DC were harvested and resuspended in an EDTA-containing medium to dislouse the clusters. Viable DC were first counted using trypan blue exclusion of dead cells. Remaining cells were stained with a variety of mouse anti-human FITC-conjugated mAbs including anti-HLA-DR (BD Biosciences, Mountain View, CA); anti-CD40,-CD80, and -CD86 (all from PharMingen); or an IgG1 isotype control (BD Biosciences) and were analyzed with a FACSscan flow cytometer (BD Biosciences). Dead cells were excluded based on side and forward scatter characteristics.

**T cell proliferation assay**

Naïve CD4⁺/CD45CD45RA⁺ T cells were isolated from adult blood buffy coats by negative depletion of cells expressing CD14, CD19, CD56, CD8, CD45RO, HLA-DR, and glycoporphin A using magnetic beads (Dynal). More than 95% of the purified cells had the CD4⁺CD45RA⁺ naïve T cell phenotype. CD11c⁺ DC were washed twice to remove any cytokine and chemokine particulate with 5 × 10⁵ 10-μl CD4⁺ T cells in round-bottom 96-well culture plates at increasing DC/T cell ratios. All cocultures were conducted in triplicate. DC alone and T cells alone were used as controls. After 5 days cells were pulsed with 1 μCi [³H]thymidine (Amersham, Arlington Heights, IL) for 16 h before harvesting and counting of radioactivity.

**Stat3 and Stat5 activation assays**

Stable Ba/F3 transfectant cells (~2.5 × 10⁶ cells) werestarved for 4–6 h and then stimulated at 10⁶ cells/ml for 15 min with either 10 ng/ml IL-3 or 30 ng/ml hTSLP. After stimulation cells were harvested and incubated for 15 min at 4°C in lysis buffer containing 50 mM Tris-HCl (pH 7.5), 300 mM NaCl, 2 mM EDTA, 0.875% Brij 97, 0.125% Nonidet P-40, 10 mg/ml aprotinin, 10 mg/ml leupeptin, 1 mM PMSF, 1 mM NaF, and 1 mM Na₃VO₄. Cell lysates were clarified by centrifugation at 12,000 × g for 15 min, and supernatants were subjected to 8% SDS-PAGE. Proteins were electrotransferred onto nylon membranes (Immobilon-P; Millipore, Bedford, MA) and detected by Western blot analysis using rabbit anti-anti-phospho-Stat3 and anti-anti-phospho-Stat5 (New England Biolabs, Beverly, MA) or anti-Stat3 and anti-Stat5 (Santa Cruz Biotechnology, Santa Cruz, CA), followed by mouse anti-rabbit Ig HRP. Immuno-reactive bands were visualized using ECL (SuperSignal West Dura Extended Duration Substrate, Pierce, Rockford, IL). Films were scanned on a Biofreeze diagnostic system (Diagnostic, Rochester, NY). For reprobing blots were stripped with 200 mM glycine and 1% SDS, pH 2.5, for 30 min at 65°C.

**Results**

Human TSLP was identified computationally

Genomic databases were searched with a computationally derived sequence profile of the IL-7 helical cytokine family using methods described by Oppmann et al. (20). This search identified an expressed sequence tag (GenBank accession no. AA889581) encoding an almost full-length novel human cytokine. A search across a panel of cDNA libraries from various tissues and cell lines identified the lung fibroblast sarcoma cell line MRC5 as a source for a full-length sequence tag (GenBank accession no. X46512) containing an almost full-length novel human cytokine. A search across a panel of cDNA libraries from various tissues and cell lines identified the lung fibroblast sarcoma cell line MRC5 as a source for a full-length sequence tag (GenBank accession no. X46512) containing an almost full-length novel human cytokine.
analysis of a large panel of human cDNAs from various libraries and cultured cell lines showed that hTSLP expression of a 1.3-kb message was restricted to a few lung libraries and several mono-cyte cell samples (data not shown).

Identification of a receptor for hTSLP

Mouse TSLP binds to a specific receptor named mTSLPR (7). This recently described molecule is most closely related to R<sub>g<sub>c</sub></sub>, the shared receptor present in the signaling complexes for IL-2, IL-4, IL-7, IL-9, IL-15, and possibly IL-21 (22). Both R<sub>g<sub>c</sub></sub> and mTSLPR sequences were used in a focused search for a candidate hTSLPR gene; consequently, a cDNA that encoded an orphan member of the hemopoietin receptor family was identified from a proprietary database (HGS, Rockville, MD). This cDNA, designated hTLSPR, contains an open reading frame encoding a 371-aa protein with a single transmembrane region (Fig. 1B). Human TSLPR displays the closest identity to mTSLPR (39%) followed by R<sub>g<sub>c</sub></sub> (24%), and most likely represents the human orthologue of mTSLPR (Fig. 1C). Intriguingly, an alternatively spliced, soluble, short form of the mTSLPR has been described (10), suggesting that an analogous human molecule could serve as a secreted inhibitor of hTSLP.

Identification of a functional heteromeric human TSLP receptor complex

The functional receptor complex for mTSLP consists of mTSLPR and mIL-7R<sub>a</sub>. To test the hypothesis that the functional receptor for hTSLP consists of hTSLPR and hIL-7R<sub>a</sub>, Ba/F3 cells were

**FIGURE 1.** Human TSLP and TLSPR sequence and evolution. A, Alignment of protein sequences for human and mouse TSLP (GenBank accession no. AF232937). The four predicted α-helices of the canonical hemopoietic cytokine fold in human and mouse TSLP are labeled A, B, C, and D. Identical residues are shaded black. B, The amino acid sequence of human TSLPR is shown in alignment with mTSLPR (GenBank accession no. AF201963). Identical residues are shaded black. The signal peptide and transmembrane domains are shown. The characteristic class I cytokine receptor motifs WSXWS and box 1 are boxed; the more typical WSXWS sequence is replaced by the sequence PSDWS in hTSLP and by PSEWT in mTSLP. C, Evolutionary dendrogram of selected short chain hemopoietic cytokines (left panel) and their receptors (human and mouse orthologues; right panel). The receptor tree is rooted by GHR (growth hormone receptor). Sequence data for human TSLP are available from GenBank under accession number AF338732. Sequence data for human TSLPR are available from GenBank under accession number AF338733.
infected with retroviral constructs encoding the receptors. While parental Ba/F3 cells would only proliferate in response to IL-3, those cells infected with either hTSLPR or hIL-7Rα alone showed no proliferative response when either hTSLP or hIL-7 was added (Fig. 2, A and B). However, Ba/F3 cells expressing both receptors proliferated strongly in response to hTSLP, but not at all upon addition of hIL-7 (Fig. 2C). The response was specific for hTSLP, as mTSLP was not able to induce proliferation (Fig. 2C). This finding establishes that the functional receptor for hTSLP consists of two subunits, hTSLPR and hIL-7Rα; furthermore, we observed no cross-reactivity between mTSLP and the hTSLP receptor complex. The corresponding activation status of Stat5 and Stat3 was also measured in the various Ba/F3 cell populations. Fig. 3 shows that both Stat5 and Stat3 were phosphorylated upon addition of hTSLP, but only when both hTSLPR and hIL-7Rα were present.

**Coexpression of TSLPR and IL-7Rα in human DC and monocytes**

To identify possible target cells capable of responding to hTSLP we have analyzed by quantitative PCR a large panel of human cDNA libraries for the simultaneous expression of both TSLPR and IL-7Rα mRNAs (Fig. 4). Several cell types expressed both receptors. Th cell clones, in particular activated DC, and to a lesser extent monocytes produced significant levels of both receptors, suggesting that these cell types might respond to TSLP.

**TSLP induces TARC on freshly isolated monocyte populations and CD11c+ blood DC**

The spectrum of biological activities induced by TSLP was investigated based on the overlapping expression patterns of TSLP receptor components. cDNA was prepared from human monocytes cultured for 24 h in the presence of TSLP or IL-7, and the expression of 38 human chemokines and 20 human chemokine receptors was analyzed by quantitative real-time PCR. Interestingly, TSLP and IL-7 influenced the expression of distinct sets of chemokines (Table I), but did not affect the expression of chemokine receptors. TSLP enhanced the expression of TARC/CCL17, DC-CK1/pulmonary and activation-regulated chemokine (PARC)/CCL18, macrophage-derived chemokine (MDC)/CCL22, and MIP3β/CCL19. IL-7 also enhanced the expression of TARC/CCL17, MDC/CCL22, and MIP3β/CCL19, but, in addition, enhanced the expression of IL-8/CXCL8, CTAPIII/CXCL7, ENA78/CXCL5, and GROαβ/CXCL12 and decreased the expression levels of IP-10/CXCL10, I-TACK/CXCL11, SDF1/CXCL12, MCP2/CCL8, and MCP4/CCL13 (Table I). The induction of TARC protein by TSLP on monocyte and DC populations was confirmed by ELISA. The level of TARC production by CD11c+ DC was at least 10-fold higher than that by monocytes (Fig. 5).

**FIGURE 2.** TSLP signaling requires TSLPR and IL-7Rα. Ba/F3 cells were infected with retroviruses encoding hTSLPR or hIL-7Rα or were coinfected with hTSLPR and hIL-7Rα constructs. Proliferation of cells upon stimulation with either IL-7 or TSLP was determined after addition of Alamar Blue and was measured at 570–600 nm. Data represent one of three experiments with similar results. The mean of triplicate determinations is shown. The parental Ba/F3 cells proliferate only in response to mIL-3 (10 ng/ml). A, Ba/F3 cells expressing IL-7Rα do not respond to IL-7 or TSLP. B, Ba/F3 cells expressing hTSLPR do not respond to IL-7 or TSLP. C, Ba/F3 cells coexpressing hTSLPR and hIL-7Rα proliferate in the presence of hTSLP, but not upon addition of hIL-7 or mTSLP. ●, mIL-3; hIL-7 or mTSLP; ○, hIL-7; △, medium.

**FIGURE 3.** TSLP induces phosphorylation of Stat3 and Stat5. Ba/F3 cells expressing hIL-7Rα, hTSLPR, or both were stimulated (see Materials and Methods) with control, mIL-3 (10 ng/ml), or hTSLP (20 ng/ml), and phosphorylation was assessed by Western blot using anti-phospho-Stat5 Ab (A) and anti-phospho-Stat3 Ab (B). Blots were reprobed using anti-Stat5 (A) and Stat3 Abs (B). Phosphorylation of Stat5 and Stat3 by hTSLP was only detected on cells expressing both receptors.
TSLP activates CD11c⁺ DC

Freshly purified immature CD11c⁺ blood DC are known to spontaneously mature in culture (23). After 24 h in medium alone, we observed loose and irregular clumps in the DC culture (Fig. 6A). In the presence of TSLP this maturation process was dramatically enhanced. DC in culture formed tight and round clumps with fine dendrites visible at the periphery of each clump (Fig. 6B). The TSLP-induced maturation was confirmed by analyzing the surface phenotype of DC using flow cytometry. Whereas TSLP slightly up-regulated the expression of HLA-DR and CD86, it strongly induced the costimulatory molecules CD40 and CD80 (Fig. 6C). This maturation process was accompanied by an increased viability of the DC (data not shown). A titration of TSLP using log dilutions of the cytokine showed that both the effect on survival and the induction of costimulatory molecules on DC were maximal at 15 ng/ml and above and were still significant at concentrations as low as 15 pg/ml (data not shown).

We next analyzed the T cell stimulatory capacity of CD11c⁺ DC cultured 24 h in medium alone or in the presence of TSLP. DCs were cocultured with 5 x 10⁴ naive CD4⁺ CD45RA⁺ allogeneic T cells at increasing DC/T cell ratios. As assessed by [³H]thymidine incorporation on day 5 of the coculture, DC cultured with TSLP induced up to 10-fold stronger naive T cell proliferation compared with DC cultured in medium (Fig. 6D).

Discussion

We describe a novel human hemopoietic cytokine and its functional receptor complex. Comparison with the recently published sequence of mouse TSLP (6) suggests that the cytokine reported here is the human orthologue of mTSLP, although the degree of identity between the two proteins is low (43%). Identification of the functional hTSLP receptor complex lends further support for the evolutionary correspondence between human and mouse TSLP. As described here, the functional hTSLP receptor consists...
The Journal of Immunology

Table I. Effects of TSLP and IL-7 on chemokine expression

<table>
<thead>
<tr>
<th>Chemokine</th>
<th>Media</th>
<th>TSLP</th>
<th>IL-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL1</td>
<td>40.0</td>
<td>1.0</td>
<td>1.4</td>
</tr>
<tr>
<td>CCL2</td>
<td>24.8</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>CCL3</td>
<td>31.5</td>
<td>0.7</td>
<td>2.0</td>
</tr>
<tr>
<td>CCL4</td>
<td>28.9</td>
<td>1.0</td>
<td>1.6</td>
</tr>
<tr>
<td>CCL5</td>
<td>30.4</td>
<td>1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>CCL7</td>
<td>31.3</td>
<td>0.7</td>
<td>1.4</td>
</tr>
<tr>
<td>CCL8</td>
<td>30.2</td>
<td>2.8</td>
<td>0.2</td>
</tr>
<tr>
<td>CCL11</td>
<td>40.0</td>
<td>1.6</td>
<td>1.4</td>
</tr>
<tr>
<td>CCL13</td>
<td>37.3</td>
<td>1.6</td>
<td>0.1</td>
</tr>
<tr>
<td>CCL14</td>
<td>40.0</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>CCL15</td>
<td>40.0</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td>CCL16</td>
<td>40.0</td>
<td>2.2</td>
<td>7.0</td>
</tr>
<tr>
<td>CCL17</td>
<td>39.8</td>
<td>195.4</td>
<td>20.1</td>
</tr>
<tr>
<td>CCL18</td>
<td>35.8</td>
<td>2.9</td>
<td>1.7</td>
</tr>
<tr>
<td>CCL19</td>
<td>36.7</td>
<td>8.5</td>
<td>8.3</td>
</tr>
<tr>
<td>CCL20</td>
<td>40.0</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>CCL21</td>
<td>40.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>CCL22</td>
<td>34.3</td>
<td>8.8</td>
<td>3.0</td>
</tr>
<tr>
<td>CCL24</td>
<td>29.3</td>
<td>2.0</td>
<td>1.2</td>
</tr>
<tr>
<td>CCL25</td>
<td>40.0</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>CCL26</td>
<td>38.9</td>
<td>1.1</td>
<td>2.5</td>
</tr>
<tr>
<td>CCL27</td>
<td>40.0</td>
<td>0.8</td>
<td>1.2</td>
</tr>
<tr>
<td>CCL28</td>
<td>40.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>CXCL1-3</td>
<td>28.7</td>
<td>1.0</td>
<td>4.4</td>
</tr>
<tr>
<td>CXCL4</td>
<td>27.9</td>
<td>1.2</td>
<td>1.9</td>
</tr>
<tr>
<td>CXCL5</td>
<td>28.7</td>
<td>1.1</td>
<td>8.0</td>
</tr>
<tr>
<td>CXCL6</td>
<td>40.0</td>
<td>1.5</td>
<td>1.3</td>
</tr>
<tr>
<td>CXCL7</td>
<td>28.7</td>
<td>1.1</td>
<td>2.0</td>
</tr>
<tr>
<td>CXCL8</td>
<td>27.3</td>
<td>1.4</td>
<td>8.5</td>
</tr>
<tr>
<td>CXCL9</td>
<td>34.9</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>CXCL10</td>
<td>29.7</td>
<td>0.6</td>
<td>0.1</td>
</tr>
<tr>
<td>CXCL11</td>
<td>32.9</td>
<td>0.7</td>
<td>0.0</td>
</tr>
<tr>
<td>CXCL12</td>
<td>33.1</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>CXCL13</td>
<td>35.4</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>CXCL14</td>
<td>39.7</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>XCL1</td>
<td>40.0</td>
<td>0.9</td>
<td>3.8</td>
</tr>
<tr>
<td>CX3CL1</td>
<td>40.0</td>
<td>1.4</td>
<td>1.3</td>
</tr>
</tbody>
</table>

* Human monocytes were cultured in the absence or presence of hTSLP (50 ng/ml) or IL-7 (50 ng/ml) for 18 h, and expression of chemokine genes was determined by quantitative PCR. Results are expressed as 1) C_T values of nonactivated samples and 2) fold difference relative to the calibrator sample (media). Boldface values indicate significant changes.

FIGURE 5. Induction of TARC by TSLP. Human monocytes (A) or CD11c^+^ DC (B) were cultured in the absence or the presence of TSLP (50 ng/ml) or IL-7 (50 ng/ml) for 24 h, and the production of TARC was determined in the culture supernatant by ELISA.
identification of a functional mTSLP-receptor complex invites a similar analysis for the present work.

Acknowledgments
We thank Mayu Almualna and Sylvia Lo for synthesizing oligonucleotides and Terri McClanahan for providing cDNA libraries.

References


