

Dichotomy of short and long thymic stromal lymphopoietin isoforms in inflammatory disorders of the bowel and skin

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Background: Thymic stromal lymphopoietin (TSLP) is a cytokine with pleiotropic functions in the immune system. It has been associated with allergic reactions in the skin and lungs but also homeostatic tolerogenic responses in the thymus and gut. **Objective:** In human subjects TSLP is present in 2 isoforms, short and long. Here we wanted to investigate the differential expression of the TSLP isoforms and discern their biological implications under homeostatic or inflammatory conditions. **Methods:** We evaluated the expression of TSLPs in tissues from healthy subjects, patients with ulcerative colitis, patients with celiac disease, and patients with atopic dermatitis and on epithelial cells and keratinocytes under steady-state conditions or after stimulation. We then tested the immune activity of TSLP isoforms both *in vitro* and *in vivo*. **Results:** We showed that TSLP isoforms are responsible for 2 opposite immune functions. The short isoform is expressed

under steady-state conditions and exerts anti-inflammatory activities by affecting the capacity of PBMCs and dendritic cells to produce inflammatory cytokines. Moreover, the short isoform TSLP ameliorates experimental colitis in mice and prevents endotoxin shock. The long isoform of TSLP is proinflammatory and is only expressed during inflammation. The isoforms are differentially regulated by pathogenic bacteria, such as *Salmonella* species and adhesive-invasive *Escherichia coli*. **Conclusions:** We have solved the dilemma of TSLP being both homeostatic and inflammatory. The TSLP isoform ratio is altered during several inflammatory disorders, with strong implications in disease treatment and prevention. Indeed, targeting of the long isoform of TSLP at the C-terminal portion, which is common to both isoforms, might lead to unwanted side effects caused by neutralization of the homeostatic short isoform. (J Allergy Clin Immunol 2015;■■■■:■■■-■■■.)

Key words: Thymic stromal lymphopoietin, gut homeostasis, skin homeostasis, anti-inflammatory drugs, ulcerative colitis, celiac disease, atopic dermatitis

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Allergic and inflammatory diseases of the skin, airways, and gut affect millions of persons worldwide, and it is becoming clear that these diseases can be pathophysiologically connected. For instance, 70% of patients with atopic dermatitis (AD) undergo development of asthma through a process called the atopic march,¹ and similarly, it has been hypothesized that exposure to food allergens through a disrupted skin barrier early in life leads to antigen sensitization and food allergy.² Hence there is a link between skin, airway, and gut inflammatory disorders.

Thymic stromal lymphopoietin (TSLP) is a cytokine involved in several physiologic and pathologic immune activities.^{3,4} TSLP has homeostatic activities because it is expressed by Hassall corpuscles in the thymus and regulates the capacity of dendritic cells (DCs)⁵ and plasmacytoid DCs⁶ to drive the development of natural regulatory T cells. TSLP can also promote the homeostatic polyclonal expansion of T cells in the absence of foreign antigen.⁷ In the gut we have shown that TSLP is expressed by intestinal epithelial cells and educates noninflammatory DCs that have reduced ability to produce IL-12p70⁸ and drive the differentiation of inducible regulatory T cells.⁹

Conversely, TSLP has been shown to play a pathogenic role in several immune disorders, including AD, asthma, and food allergy.⁴ Indeed, TSLP can drive the development of strong allergic T_H2 responses with release of IL-4, IL-5, IL-13, and TNF- α ¹⁰ through upregulation of OX40 ligand expression on TSLP-treated DCs.¹¹ TSLP is overexpressed in the airways of

Abbreviations used

AD:	Atopic dermatitis
CD:	Celiac disease
DC:	Dendritic cell
DSS:	Dextran sodium sulfate
ITSLP:	Long isoform thymic stromal lymphopoietin
MAPK:	Mitogen-activated protein kinase
mDC:	Myeloid dendritic cell
moDC:	Monocyte-derived dendritic cell
NF- κ B:	Nuclear factor κ B
poly I:C:	Polyinosinic:polycytidylic acid
shTSLP:	Short isoform thymic stromal lymphopoietin
STAT5:	Signal transducer and activator of transcription 5
TLR:	Toll-like receptor
TSLP:	Thymic stromal lymphopoietin
TSLPR:	Thymic stromal lymphopoietin receptor
UC:	Ulcerative colitis

asthmatic patients and is associated with T_H2 cytokines.^{12,13} TSLP is directly involved in the itch response,¹⁴ in activating innate lymphoid cells to induce skin inflammation,¹⁵ and in the atopic march.¹⁶ Consistently, TSLP polymorphisms are linked to asthma susceptibility¹⁷⁻¹⁹ and AD.^{20,21} TSLP influences the differentiation of basophils, which are more prone to promote T_H2 inflammation²² and involved in eosinophilic esophagitis.²³

Hence it seems that TSLP has both physiologic and pathologic activities that are difficult to reconcile. Here we show that this dichotomy can be explained by the presence of 2 distinct isoforms of TSLP, a long and a short one, which are differentially regulated and involved in inflammatory and anti-inflammatory responses, respectively.

METHODS**Tissue sampling**

Intestinal mucosa was excised from the intestines of patients with ulcerative colitis (UC) at the time of surgery ($n = 13$). As a control, intestinal samples were obtained from healthy tissue (at least 7 cm away from neoplastic tissue) of patients undergoing surgery for colon cancer ($n = 26$). The mucosal layer was separated from the rest of the tissue by a pathologist and directly transferred to our laboratory. Transfer was carried out in both cases in HBSS buffer at 4°C supplemented with bacteriostatic antibiotics. Mucosa from patients with celiac disease (CD) was obtained by means of biopsy during routine colonoscopy (patients with treated CD, $n = 15$; patients with untreated CD, $n = 13$; healthy subjects, $n = 13$). Skin biopsy specimens were taken from patients with AD; lesional, nonlesional tissue, and healthy tissue (from the back or the thigh) was collected ($n = 24$). The clinical diagnosis was confirmed by means of dermatopathologic evaluation. All tissues were obtained from patients who signed an informed consent form approved by the institutional review board allowing material not required for diagnosis to be used for research purposes. The samples were handled in a completely anonymous manner and assigned a serial number by the pathologist for cross-checking histologic reference.

On the basis of a series of preliminary experiments, the size of samples for each disease group has been calculated to estimate the statistical differences with a power of 0.80 and an α value of .05.

Mice

Female C57Bl/6J mice (8-10 weeks of age) were obtained from Charles River Laboratories (Milan, Italy). Mice were bred and maintained at the IFOM-IEO Campus Animal Facility under specific pathogen-free conditions. All experiments were performed in accordance with the guidelines established in the Principle of Laboratory Animal Care (directive 86/609/EEC).

LPS-induced endotoxic shock

Mice were treated intraperitoneally with short isoform TSLP (shTSLP) at 50, 100, and 200 μ g per mouse in 200 μ L of injectable water twice (18 hours and 2 hours) before LPS administration ($n = 8$ per group). Control mice received water. LPS (*Escherichia coli* serotype 026:B6; Sigma-Aldrich, St Louis, Mo) was injected intraperitoneally at 200 μ g per mouse in 200 μ L of injectable water. After 6 hours, mice were killed by means of exsanguination after achievement of anesthesia, and blood was collected. IFN- γ , IL-6, and IL-12p40 levels were detected in serum by means of ELISA (R&D Systems, Minneapolis, Minn).

Dextran sodium sulfate-induced colitis

Colitis was induced by adding 3% (wt/vol) dextran sodium sulfate (DSS; TdB Consultancy AB, Uppsala, Sweden) to drinking water for 9 days. Mice were treated intraperitoneally with shTSLP (200 μ g in 200 μ L of injectable water) the day before DSS administration and every other day during the entire study ($n = 12$ per group). Mice were monitored daily for weight loss.

Bacterial strains

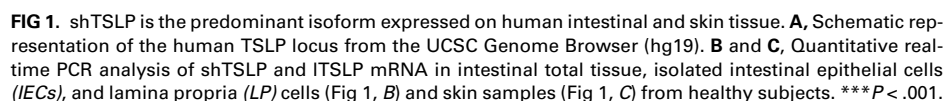
Salmonella enterica serovar typhimurium SL1344 strain FB62 (hereafter referred to as *Salmonella* typhimurium) and *E coli* strains LF82 and MG1655 (a kind gift from Arlette Darfeuille-Michaud) were cultured in TB broth. For infection, bacteria were grown at 37°C under agitation until reaching the exponential growth phase (OD between 0.55 and 0.65). Colony-forming unit numbers were controlled by means of plating.

Statistical analysis

All statistics were performed with a 2-tailed Student *t* test or 1-way ANOVA with GraphPad Prism software (GraphPad Software, La Jolla, Calif). Statistical significance was retained for *P* values of less than .05. When results are reported in terms of fold increase, statistical tests were applied to log-transformed data.

RESULTS**shTSLP is the homeostatic isoform of TSLP and is constitutively expressed in healthy skin and gut**

We carried out an analysis on the UCSC Genome Browser of human TSLP isoforms. There are 3 transcript variants (RefSeq database), but only 2 give rise to coding RNAs: the canonical TSLP transcript variant 1 (NM_033035_hg19 160 chr5:110407589-110411772) and a transcript variant 2 (NM_138551_hg19 64 chr5:110409281-110411772). The 2 coding transcripts code for the long isoform of thymic stromal lymphopoietin (ITSLP) of 159 amino acids (variant 1) and for shTSLP (variant 2), which encompasses the last 63 residues of ITSLP and is identical to its C-terminal portion (Fig 1, A). They are not alternatively spliced isoforms but derive from the activity of 2 putative promoter regions (Fig 1, A). In Fig 1, A, we portrayed the ENCODE track profiles showing active enhancers (H3K4me1 high, H3K4me3 low, and H3K27ac high) or promoters (H3K4me1 low, H3K4me3 high, and H3K27ac high) in the middle, and at the bottom, we portrayed the ENCODE DNaseI hypersensitive site clusters derived from 125 cell types and transcription factor binding regions assessed by using ChIP-seq experiments. This analysis highlights that although the region upstream from the long isoform under steady-state conditions is hardly used in most of the cell lines present in the UCSC database, the short isoform promoter shows a high capacity to bind a number of different transcription factors (Fig 1, A). Hence we assessed the expression of the 2 isoforms in healthy skin and gut compartments. We designed primers specific for either ITSLP or



The molecular pathway of ITSLP signaling is well characterized; it mediates its effects after binding to its own receptor, a complex formed by thymic stromal lymphopoietin receptor (TSLPR) and the IL-7 receptor α chain,^{24,25} and phosphorylation of signal transducer and activator of transcription 5 (STAT5) is a key downstream event.²⁶ To elucidate the pathways of shTSLP signaling, we used a human phosphokinase array that analyzes phosphorylation of 46 intracellular kinases (Human Phospho-Kinase Array, R&D Systems) after 5 and 20 minutes of stimulation (Fig 3, A). shTSLP induced phosphorylation of a limited number of molecules, including p38 α , extracellular signal-regulated kinase 1/2, and Lyn, but had only a mild effect on STAT5 phosphorylation (Fig 3, A). The capacity of shTSLP to activate p38 α was confirmed by means of Western blotting in 4 different moDC donors (Fig 3, B). We then performed the same kinase array on LPS-stimulated moDCs previously conditioned or not with shTSLP to assess whether it was modulating Toll-like receptor (TLR)-mediated signaling. Interestingly, the level of p38 α and extracellular signal-regulated kinase 1/2

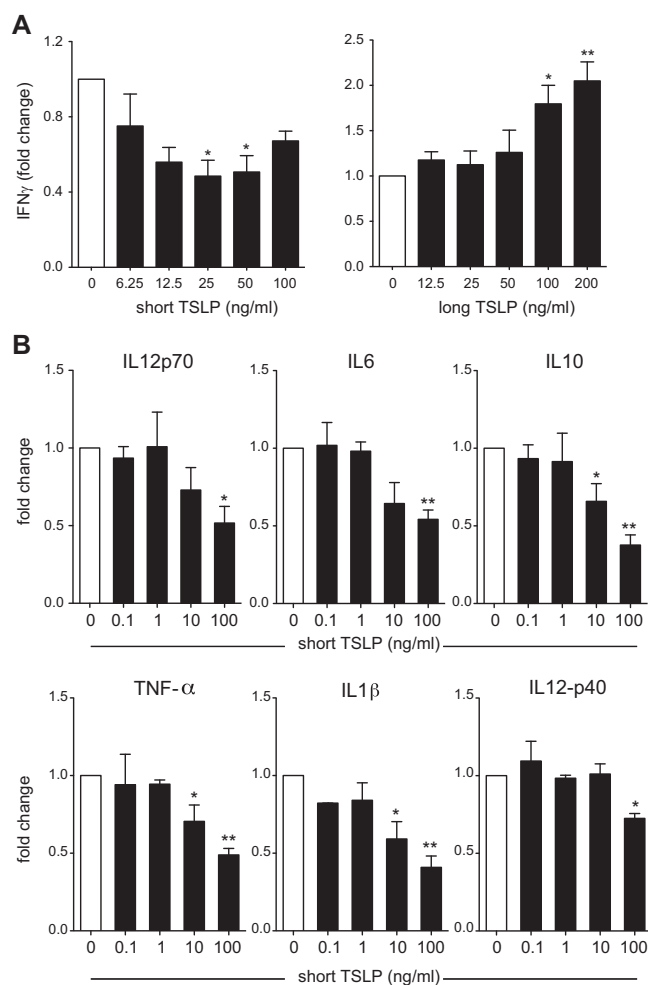


FIG 2. shTSLP has anti-inflammatory properties. **A**, IFN- γ production in a bidirectional mixed lymphocyte reaction in the presence of shTSLP and ITSLP. Results of 3 independent experiments are shown. **B**, Inflammatory cytokine secretion of mDCs conditioned with shTSLP and then infected with *Salmonella typhimurium*. Results of 4 independent experiments are shown. Data are expressed as a "fold change" considering untreated PBMCs or cells treated only with *Salmonella typhimurium* as a control (value = 1). Significant differences were determined by means of 1-way ANOVA with the Dunnett test. * $P < .05$ and ** $P < .01$.

phosphorylation was decreased when DCs were preconditioned with shTSLP (see Fig E3 in this article's Online Repository at www.jacionline.org), suggesting that it affects LPS-mediated mitogen-activated protein kinase (MAPK) activation. We could speculate that the slight phosphorylation of p38 MAPK induced by shTSLP might either desensitize the cells against further activation or raise the threshold of mDC activation, thus resulting in decreased cytokine production after TLR engagement.

mDCs do not express TSLPR, and thus to test the capacity of both isoforms to induce TSLPR-mediated signaling, we switched to myeloid blood-isolated CD1c⁺ DCs that are TSLPR⁺. We found that only ITSLP induced STAT5 phosphorylation (Fig 4, A). This did not exclude the possibility of shTSLP binding to TSLPR and acting as an antagonist, blocking signal from the ITSLP. However, when myeloid dendritic cells (mDCs) were stimulated with the 2 isoforms, either alone or together in an equimolar ratio, shTSLP had no capacity to block or inhibit ITSLP-dependent STAT5 phosphorylation (Fig 4, A). This suggests that shTSLP does

not bind to TSLPR and is not dominant negative. Indeed, only ITSLP significantly upregulated CCL17 and CCL22 expression, as expected from TSLP activation,¹⁰ and this was not affected by the presence of shTSLP (Fig 4, B). Consistently, ITSLP-conditioned mDCs were inflammatory and significantly upregulated secretion of TNF- α from naive T cells in an allogeneic mixed lymphocyte reaction, both in the presence and absence of shTSLP (Fig 4, C). However, shTSLP is capable of exerting an anti-inflammatory activity on mDCs because shTSLP-conditioned mDCs were impaired in inducing IFN- γ secretion by T cells during the mixed lymphocyte reaction (Fig 4, D), a property not shared by ITSLP (data not shown), indicating that mDCs respond to shTSLP in an anti-inflammatory way.

shTSLP prevents endotoxin shock and ameliorates experimental colitis *in vivo*

In the mouse a short TSLP isoform has not been described or annotated in RefSeq. However, the C-terminal region of mouse TSLP shares 40% identity with human shTSLP (see Fig E4 in this article's Online Repository at www.jacionline.org). Thus we analyzed whether human shTSLP might have anti-inflammatory properties also in the mouse *in vivo*. To this end, C57/BL6 mice were injected intraperitoneally with shTSLP 18 and 2 hours before injection with LPS. Six hours after LPS injection, mice were killed, and cytokines were measured in the sera. Prophylactic injection with shTSLP led to a significant decrease of IL-6, IL-12/23p40, and IFN- γ levels in a dose-dependent fashion (Fig 5, A). Because we found that in the human gut TSLP is predominant at steady-state conditions, we assessed whether it might exert a protective role during gut perturbations, such as during experimental colitis. We administered shTSLP to mice every other day to evaluate whether it could protect against DSS-induced colitis. Mice treated with 200 μ g of shTSLP displayed significantly reduced weight loss and faster recovery (Fig 5, B), suggesting a protective role during gut-induced inflammation.

Taken together, these data support a homeostatic/anti-inflammatory role of shTSLP *in vitro* and *in vivo*.

Short/long TSLP ratios are affected in gut disorders

We previously described that intestinal epithelial cells isolated from patients with Crohn disease have lost the ability to express TSLP by using primers that do not distinguish between the 2 isoforms.^{8,9} Knowing that ITSLP is not expressed under steady-state conditions (Fig 1, B), we now know that the reduction in TSLP expression was attributable to the lack of shTSLP expression. Hence we evaluated whether there was a deregulation of TSLP expression in patients with other inflammatory bowel disorders. We analyzed the expression of TSLP isoforms in cells isolated from patients with UC and in total tissue biopsy specimens from patients with CD (see Table E2 in this article's Online Repository at www.jacionline.org). We found that ITSLP and TSLPR were significantly upregulated in tissues from patients with UC compared with levels seen in healthy colonic mucosa, whereas shTSLP expression was unchanged (Fig 6, A, and see Fig E5, A, in this article's Online Repository at www.jacionline.org). These data were confirmed at the protein level through immunofluorescence staining and Western blotting with an antibody directed specifically to ITSLP. We generated

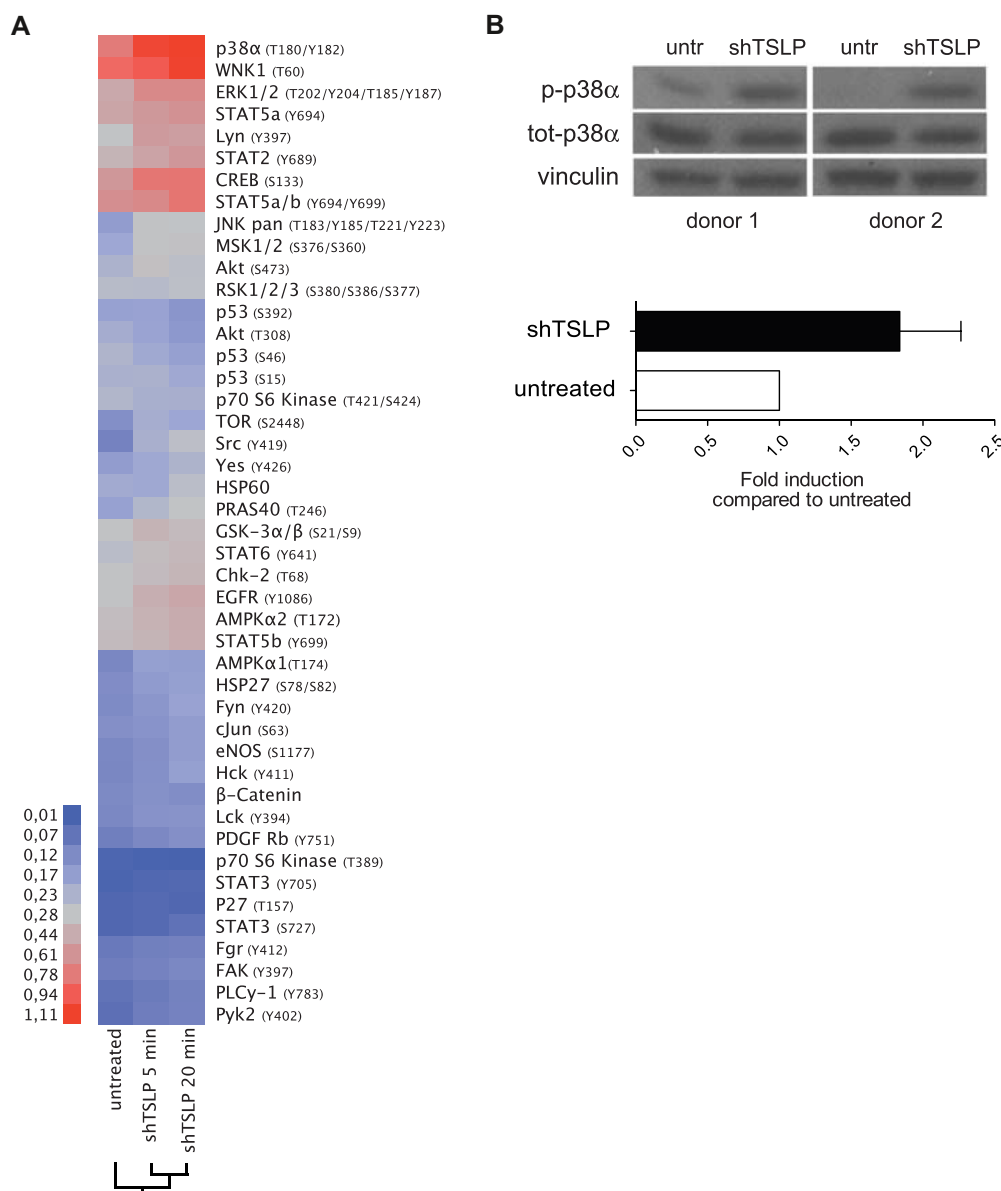


FIG 3. shTSLP increases p38α phosphorylation in moDCs. **A**, Human phosphokinase array assay of moDCs left untreated or conditioned for 5 and 20 minutes with 100 ng/mL short TSLP. **B**, Quantification of p38α phosphorylation in untreated and shTSLP-stimulated moDCs by means of Western blotting: representative membranes from 2 donors (*upper panel*) and quantification analysis of 4 independent donors (*lower panel*). Values are normalized against total p38α protein levels and compared with untreated cells.

a polyclonal antibody targeted to the N-terminal region of ITSLP that is not present in shTSLP (see Fig E1). This antibody did not detect any protein in healthy tissue sections, whereas it stained positive on biopsy specimens from patients with UC (Fig 6, B) both in the isolated epithelial cell and lamina propria compartments. By contrast, an antibody that recognizes both isoforms of shTSLP (Fig 6, B). We used the same antibodies for Western blot analysis of protein lysates from biopsy specimens of healthy subjects (n = 9) and patients with UC (n = 9). The antibody that recognizes both isoforms can be used to identify shTSLP based on its molecular weight. We confirmed that ITSLP expression is greatly upregulated in patients with UC. shTSLP is present in tissues from both healthy subjects and patients with UC, and

interestingly, it shows one additional band (about 6 KDa), probably because of cleavage, degradation, or both, particularly in UC samples (see Fig E6 in this article's Online Repository at www.jacionline.org). Hence in healthy conditions the only isoform expressed is the shTSLP, whereas under inflammation, the ITSLP appears.

We then analyzed the expression of the 2 isoforms in tissue from patients with CD either untreated or under a gluten-free diet (treated). In this case both isoforms were significantly down-regulated in patients with untreated CD compared with those with treated CD and biopsy specimen from healthy subjects (Fig 6, C). TSLPR expression instead was not modulated between the groups (see Fig E5, B). The data are referred to cytokeratin 18 expression to take into consideration a possible reduction in the number of

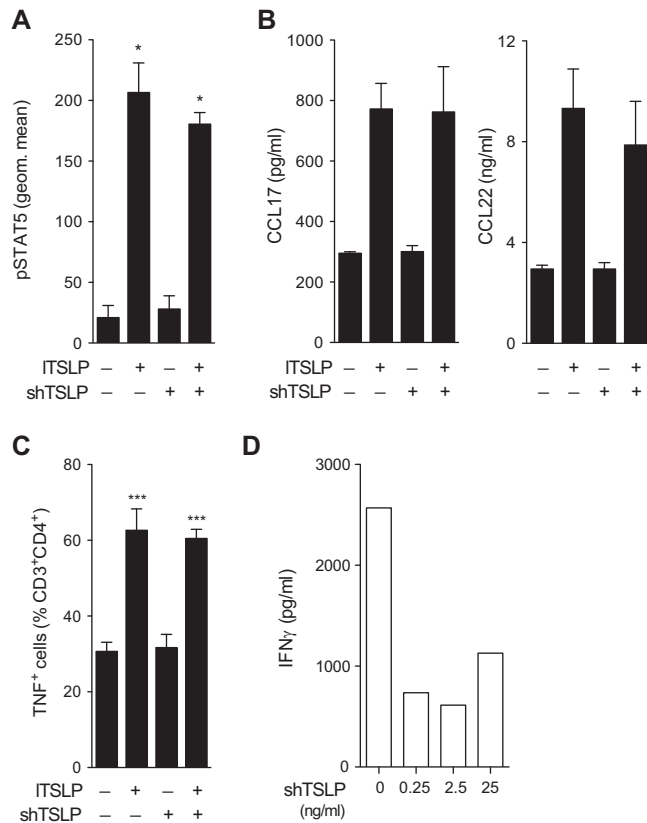


FIG 4. Effects of shTSLP on mDCs *in vitro*. **A**, Fluorescence-activated cell sorting analysis of phospho-STAT5 on mDCs left untreated or incubated with shTSLP, ITSLP, or both. **B**, ELISA of CCL17 and CCL22 levels in supernatants of mDCs treated as in Fig 4, **A**. Results of 2 independent experiments are shown. The same cells were washed and cultured with naive CD4⁺ T cells. **C** and **D**, Intracellular staining for TNF (Fig 4, **C**) and detection of IFN- γ in supernatants (Fig 4, **D**) were performed after 6 days of coculture. Fig 4, **C**, Results of 4 independent experiments are shown. Fig 4, **D**, Data show a representative of 3 independent experiments. Significant differences were determined by using 1-way ANOVA with the Tukey test. In Fig 4, **A** and **C**, a significant increase over untreated cells is indicated by asterisks. * $P < .05$ and *** $P < .001$.

epithelial cells in untreated patients. This indicates that intestinal inflammatory disorders are characterized by an alteration of the ratio between shTSLP and ITSLP.

TSLP isoform expression in epithelial cells is regulated by proinflammatory stimuli

Given the divergent properties of TSLP isoforms, we analyzed whether there were conditions that affected TSLP expression and that could be of relevance to the inflammatory gut disorders in which the ratio of the 2 isoforms is altered. We first assessed basal levels of expression of the 2 isoforms by the intestinal epithelial cell line Caco-2 using real-time PCR, and we noticed an identical trend to the one we observed in primary intestinal epithelial cells (see Fig E7, **A**, in this article's Online Repository at www.jacionline.org). We confirmed these data also at the protein level by means of Western blot analysis on noninfected Caco-2 cells (see Fig E7, **B**). However, this pattern was completely reversed when Caco-2 cells were challenged with *Salmonella* typhimurium. In this case a significant upregulation of ITSLP expression, accompanied by a downregulation of shTSLP expression, was

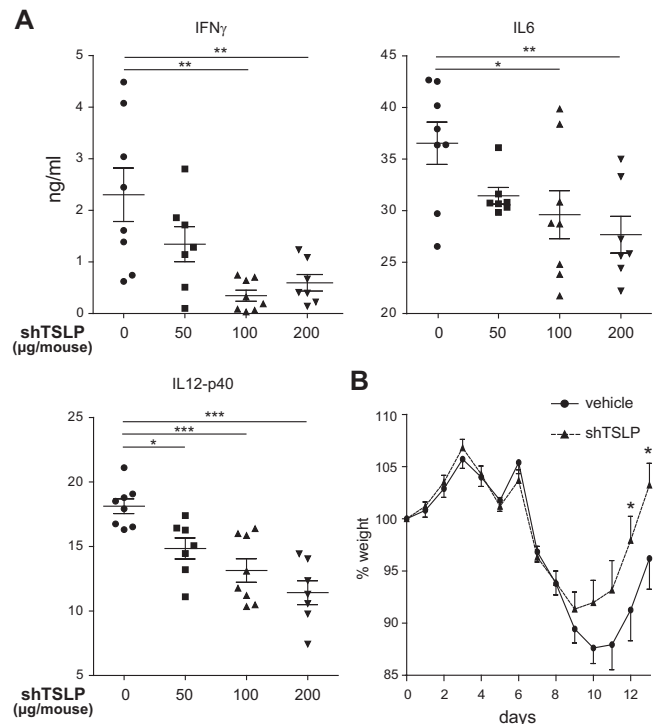


FIG 5. shTSLP has anti-inflammatory effects *in vivo*. **A**, Cytokine levels in the sera of mice treated intraperitoneally with shTSLP 18 hours and 2 hours before inducing LPS shock. Blood was collected 6 hours later. Results of 2 independent experiments are shown. Significant differences were determined by using 1-way ANOVA with the Dunnett test. **B**, Body weight changes in mice with DSS-induced colitis treated with shTSLP or vehicle intraperitoneally. The graph shows the percentage of body weight relative to initial body weight. * $P < .05$, *** $P < .01$, and **** $P < .001$.

observed (Fig 6, **D**). A similar trend of ITSLP expression upregulation was observed when we used the adherent-invasive *E coli* strain LF82, although this did not reach statistical significance (Fig 6, **D**). Interestingly, the *E coli* nonpathogenic strain MG1655 had no effect on both isoforms.

TSLP isoforms are deregulated in patients with AD and skin keratinocytes exposed to inflammatory stimuli

When we analyzed the expression of TSLP isoforms in skin inflammatory disorders, such as AD, we observed an even more complex picture. As expected from the literature,¹⁰ we found an upregulation of the ITSLP isoform in lesional as opposed to non-lesional biopsy specimens (Fig 7, **A**); unexpectedly, we found that shTSLP was significantly downregulated in lesional biopsy specimens (Fig 7, **A**). No difference was observed in TSLPR expression (see Fig E5, **C**). Immunofluorescence staining of tissue sections with the 2 antibodies confirmed the results obtained by means of qPCR, even though technically we could not demonstrate a reduction in the expression of shTSLP in lesional skin (Fig 7, **B**). This indicates that we have a further unbalance of the 2 isoforms in patients with AD because we find a concomitant downregulation of shTSLP and upregulation of ITSLP.

We then examined the effect of proinflammatory and anti-inflammatory stimuli in the 2 isoforms' expression on human primary keratinocytes, HEKa cells. Vitamin D deficiency has been

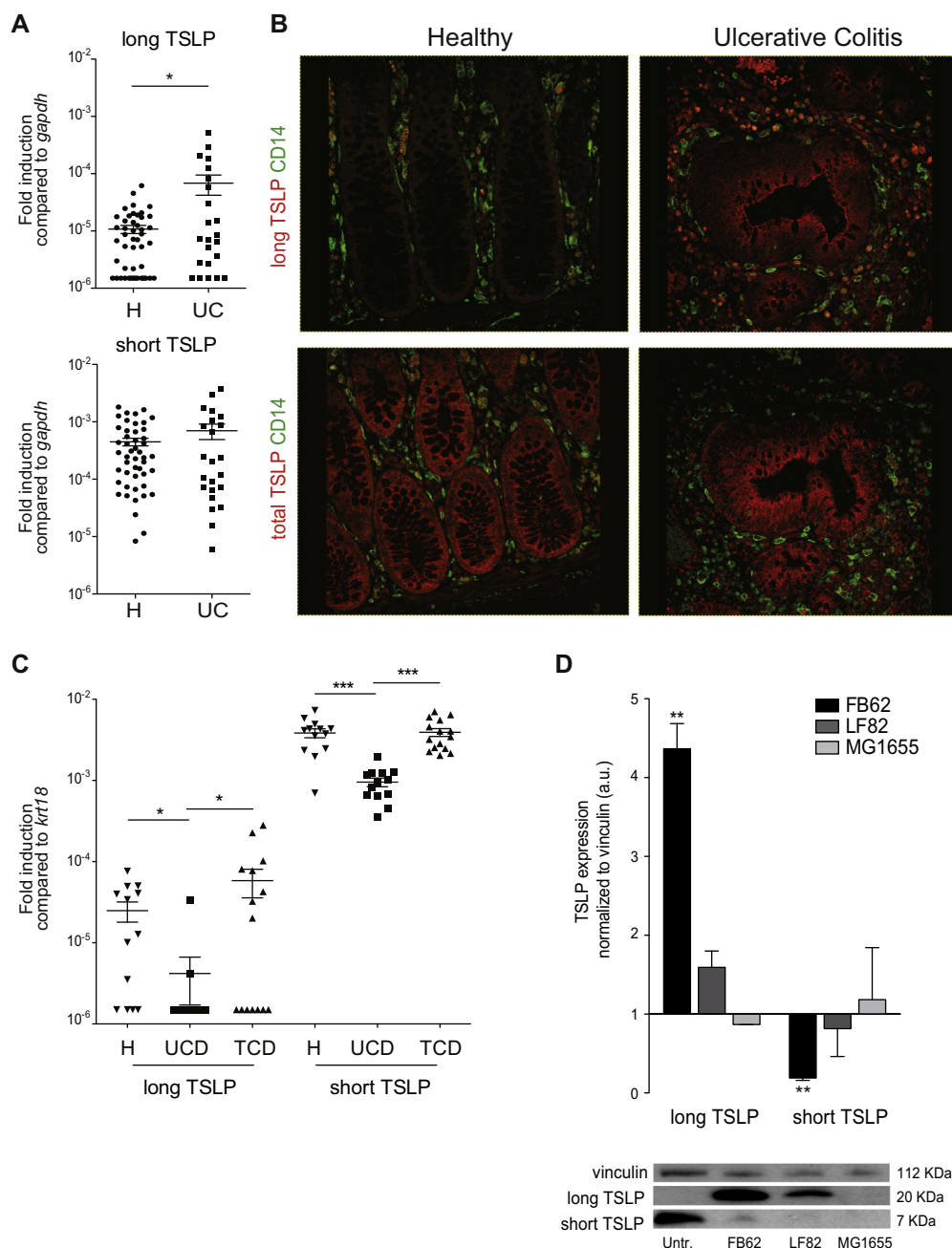


FIG 6. TSLP isoforms are differentially expressed in the intestines of patients with UC and those with CD compared with healthy subjects and differentially modulated in epithelial cells. **A** and **C**, Quantitative PCR analysis of shTSLP and ITSLP in epithelial and LP cells from tissues of healthy subjects (*H*; *n* = 26) patients with UC (*n* = 13; Fig 6, **A**) or biopsy specimens of patients with untreated CD (*UCD*; *n* = 13), patients with treated CD (*TCD*, *n* = 15), patients with CD, and healthy subjects (*n* = 13; Fig 6, **C**). **B**, Representative immunofluorescent stainings of ITSLP (upper panels, red), both TSLP isoforms (bottom panels, red), and CD14⁺ cells (green) in sections of colon. **D**, Quantification of TSLP isoforms protein expression in untreated or infected Caco-2 cells. **P* < .05, ***P* < .01, and ****P* < .001.

associated with AD severity in children^{27,28} and adult Koreans.²⁹ Furthermore, putative vitamin D response elements have been found in the upstream region of ITSLP³⁰ in addition to the well-known nuclear factor κ B (NF- κ B)-responsive element that allow its expression in response to TLR ligands.³¹ Thus we challenged HEK293T cells, which were previously starved for 24 hours, with either vitamin D3 (10 μ M/L) or polyinosinic:polycytidylic acid (poly I:C; 5 μ g/mL), a TLR3 agonist, and we performed chromatin

immunoprecipitation and quantitative PCR analysis of H3K27 acetylated long and short promoter regions. We observed that the promoter of shTSLP was more active than that of ITSLP in untreated cells (Fig 7, **C**). Interestingly, vitamin D3 induced enrichment in H3K27Ac in the promoter of both ITSLP and shTSLP, resulting in increased transcription primarily of the shTSLP isoform (Fig 7, **C** and **D**). On the other hand, poly I:C induced H3K27ac deposition only in the promoter of ITSLP, and this resulted in a stronger

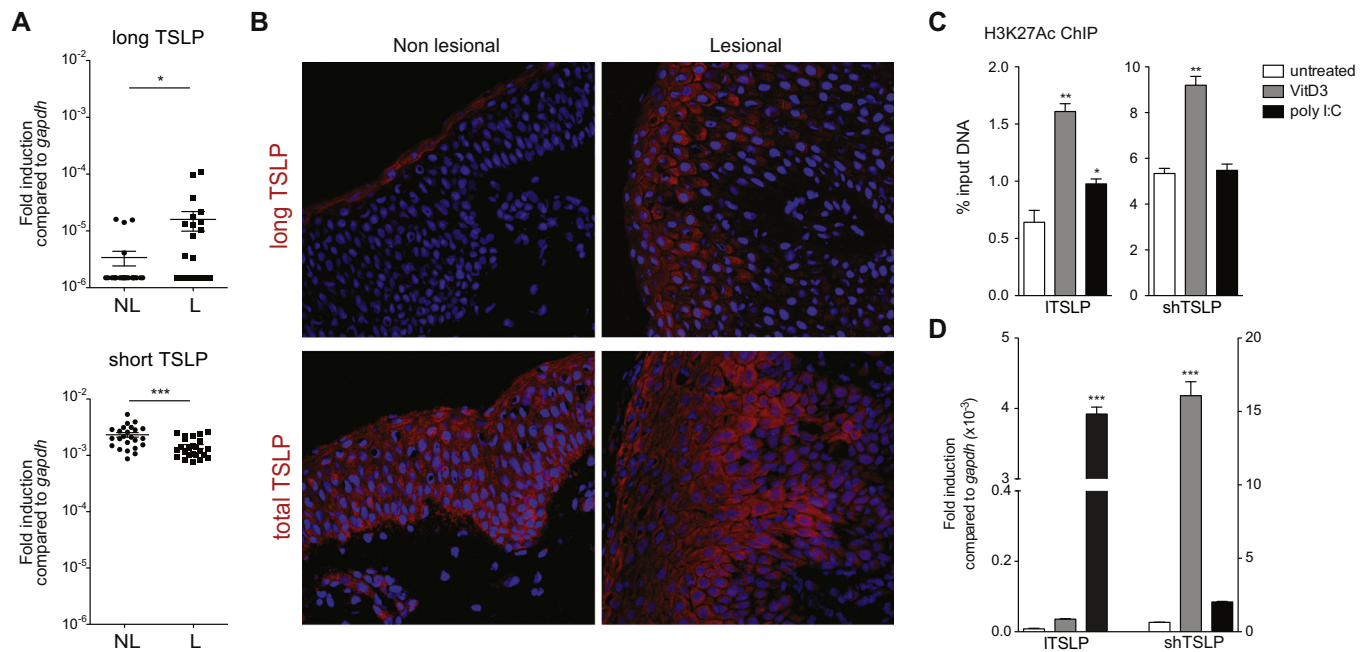


FIG 7. shTSLP and ITSLP are differentially expressed in skin tissue of patients with AD. **A**, Quantitative PCR analysis of shTSLP and ITSLP in skin biopsy specimens from nonlesional (NL) and lesional (L) skin of patients with AD ($n = 24$). **B**, Representative immunofluorescent staining of ITSLP (upper panels, red) and both TSLP isoforms (bottom panels, red) in tissue sections of nonlesional and lesional skin. **C** and **D**, ChIP-quantitative PCR analysis of H3K27Ac-enriched regions in the promoters of the TSLP isoforms (Fig 7, C) and mRNA levels of TSLP isoforms (Fig 7, D) in untreated HEK293T or those stimulated with vitamin D3 ($10 \mu\text{mol/L}$) and poly I:C ($5 \mu\text{g/mL}$). * $P < .05$, ** $P < .01$, and *** $P < .001$.

induction of ITSLP mRNA compared with vitamin D3 stimulation, even though the acetylation level was lower (Fig 7, C and D). This indicates that poly I:C and presumably NF- κ B activate only the ITSLP promoter and result in strong ITSLP mRNA transcription. By contrast, vitamin D3 activates both regions and induces shTSLP transcription but is not sufficient for ITSLP transcription. A similar result was obtained with the keratinocyte cell line HaCaT (see Fig E8 in this article's Online Repository at www.jacionline.org) and is consistent with the literature on primary keratinocytes, where an up-regulation of total TSLP mRNA, but not ITSLP mRNA, was shown in response to vitamin D3.³² Thus we can conclude that isolated epithelial cells and skin keratinocytes express ITSLP only in response to invasive bacteria or proinflammatory stimuli, whereas expression of shTSLP is constitutive but can be upregulated by anti-inflammatory mediators (vitamin D3) or downregulated by pathogenic bacteria (*Salmonella typhimurium*).

DISCUSSION

Here we have shown that the dilemma of TSLP mediating proinflammatory and anti-inflammatory activities can be explained by the existence of 2 isoforms of TSLP that are not alternatively spliced, but each one is driven by a differentially regulated promoter region. At the protein level, shTSLP encompasses the last 63 residues of ITSLP. Consistent with a higher activity of shTSLP promoter in untreated cells, shTSLP is the primary isoform expressed under steady-state conditions and is anti-inflammatory and homeostatic, whereas ITSLP is pathogenic and induced by inflammatory stimuli. shTSLP does not seem to have antagonistic activity on ITSLP but can condition both mDCs and moDCs to limit their inflammatory potential, probably by inducing a suboptimal

activation of MAPK, at least on moDCs. Consistently, shTSLP does not induce or inhibit signaling through the receptor of ITSLP, suggesting a signaling pathway that is independent on TSLPR, which is not expressed on moDCs at steady-state conditions.

The existence of a parallel and molecularly independent mechanism underlying the anti-inflammatory functions of shTSLP is fully supported by structural analysis of the murine TSLP-TSLPR-IL-7 receptor α complex.³³ Mouse TSLP, which is the ortholog of human ITSLP, is 140 residues long and folds as a 4-helix bundle, stabilized by 3-disulphide bonds and several hydrophobic interactions contributed by the N-terminal portion of the protein. Because this region is not present in shTSLP, we predict that shTSLP adopts a significantly different conformation than ITSLP, which might explain why it uses a different receptor. The 3-dimensional structure of mouse and human ITSLP is likely conserved because most of the residues involved in the scaffold of TSLP are conserved³³ and there is a 40% amino acid identity in the sequences of the regions corresponding to shTSLP between mice and human subjects. Hence even though in the mouse there is no open reading frame for shTSLP, this protein might still be generated by protease cleavage from ITSLP. Hence it is not surprising that, when tested in mice, human shTSLP could protect them from endotoxin shock and DSS colitis, suggesting an anti-inflammatory role *in vivo*.

The 2 isoforms are modulated during inflammation. In particular, invasive pathogenic bacteria, such as *Salmonella typhimurium* or adherent-invasive *E coli* strain, can upregulate the expression of the ITSLP in intestinal epithelial cells, whereas *Salmonella typhimurium* is also capable of downregulating shTSLP. Because *E coli* with adhesive and invasive capacities has been associated with inflammatory bowel disease,^{34,35} this might explain why there is an

upregulation of ITSLP in patients with UC. By contrast, vitamin D3 can activate the promoter and upregulate the expression of shTSLP in skin keratinocytes. This is an important observation because we found shTSLP expression to be downregulated in patients with AD, and a negative correlation between vitamin D3 levels and the severity of AD has been shown.²⁷⁻²⁹ Hence one of the mechanisms of action of vitamin D3 supplementation, which has been shown to be beneficial in the treatment of AD,³⁶ might be through the re-establishment of a homeostatic condition through upregulation of the shTSLP. In patients with CD, we found a downregulation of TSLP levels that are normalized when patients are on a gluten-free diet, indicating that this is probably not a primary defect in these patients.

Interestingly, because we found that shTSLP is associated with the control of inflammatory mediator release both *in vitro* and *in vivo* and with a reduction of IFN- γ production, we can hypothesize that conditions leading to a reduction in shTSLP expression could lead to an uncontrolled T_H1 type of responses, such as in patients with CD. By contrast, when ITSLP is upregulated, such as in patients with AD and UC, presumably through NF- κ B activation, which is a hallmark of inflammation in both inflammatory bowel disease and AD,³⁷⁻³⁹ a T_H2 component is induced. In addition, 2 recent reports have shown an antimicrobial activity of peptides within the shTSLP sequence, indicating a possible additional homeostatic activity of shTSLP *in vivo*.^{40,41} Hence in a personalized view of treatment for patients with inflammatory disorders, one should take these 2 aspects into account and might on the one hand block the inflammatory potential of ITSLP when this is upregulated and on the other hand re-establish immune homeostasis through administration of shTSLP or drugs involved in its upregulation, when shTSLP is downregulated. Additionally, caution should be taken when using in therapy antibodies or biologicals that do not distinguish between the 2 isoforms and that might elicit unwanted effects by targeting shTSLP.

Key messages

- Human TSLP exists in 2 distinct isoforms: shTSLP and ITSLP.
- shTSLP is the only isoform expressed in healthy skin and gut tissue, whereas ITSLP is present in diseased inflammatory conditions.
- The shTSLP/ITSLP ratio is altered in patients with UC, CD, and AD.
- shTSLP, contrary to ITSLP, has an anti-inflammatory effect both *in vitro* and *in vivo*.
- The dual role of TSLP isoforms could explain the literature paradox of human TSLP being both proinflammatory and tolerogenic.

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1 **Supplementary data**

2

3 **Materials and methods**

4 **Isolation of intestinal epithelial cells (IEC) and lamina propria (LP) cells**

5 Upon arrival in the laboratory intestinal mucosa was processed for IECs and LP cells isolation as
6 previously described ^{E1}. Briefly, the tissue was cut into small pieces and washed twice in HBSS
7 (Euroclone), 1% fetal bovine serum (FBS, Gibco), 1mM DTT for 15 min at 37°C to remove the
8 mucus. IECs were then detached by washing with HBSS, 1% FBS, 1mM EDTA for additional 15
9 minutes. IECs were pelleted and lysed with Trizol reagent (Ambion, Invitrogen) for RNA
10 extraction. The rest of the tissue was cut up in 1mm² pieces and digested in α-MEM with 5% FBS,
11 in the presence of collagenase VIII (1 mg/mL, Sigma-Aldrich), DNase I (5 U/mL, Roche
12 Diagnostics), 100 IU/ml penicillin and 100 µg/ml streptomycin for 10 min at 37°C by gentle
13 shaking ^{E2}. LP cell pellet was lysed in Trizol reagent.

14

15 **RNA extraction and RTPCR**

16 RNA was extracted using the RNeasy Micro Kit (Qiagen). RNA was reverse transcribed with
17 oligo(dT) and ImProm-II™ Reverse Transcriptase (Promega). Quantitative real-time PCRs were
18 performed with Fast SYBR Green PCR kit on the Applied Biosystems 7900HT Fast RT-PCR
19 System (Applied Biosystems). Results were quantified using the 2- Ct method ^{E3}. Results are
20 expressed as ‘fold induction’ in reference of the expression of the housekeeping gene, *gapdh* or
21 *krt18*. Primer list is reported in Supplemental Table 1.

22

23 **Reagents**

24 TSLP recombinant protein, long TSLP (aa 29-159, mw 15KDa, Uniprot: Q969D9), was purchased
25 from R&D Systems.

26

27 **TSLP expression in baculovirus system and purification**

28 The cDNAs encoding long (aa 29-159) and short (aa 4-60) form of human TSLP were generated by
29 PCR and subcloned into BamHI/SalI site of pFL-GST transfer vector (Invitrogen), and the resulting
30 plasmid was used for expression in insect cells (Sf9 and Hi5 cells). After 3 days infected insect cells
31 were resuspended in lysis buffer (20 mM Tris [pH 7.4], 300 mM NaCl, 5% glycerol, 1mM EDTA,
32 1mM DTT and protease inhibitors), sonicated gently and the lysates were cleared by centrifugation.
33 TSLP fragments were absorbed to Gluthation Sepharose Beads (Amersham) and eluted using lysis
34 buffer supplemented with 20 mM reduced Gluthation (Sigma). The eluted proteins were dialysed in
35 buffer containing 20 mM Tris [pH 7.4], 200 mM NaCl, 5% glycerol, 1mM EDTA, 1mM DTT. As
36 the expression of shTSLP in baculovirus led to low amounts of the protein, not compatible with in
37 vivo studies, we chemically synthesized the peptide and found to have similar anti-inflammatory
38 properties (not shown).

39

40 **Short TSLP peptide synthesis**

41 Short TSLP (aa 4-60, mw 7KDa, Uniprot: Q96AU7) was synthesized in house and resuspended in
42 sterile water. Peptide was identified by MALDI-TOF mass spectrometry analysis. Endotoxin levels
43 were below 0.1 ng/μg of peptide as determined by LAL test.

44 The peptide was assembled by stepwise microwave-assisted Fmoc-SPPS on a Biotage ALSTRA
45 Initiator+ peptide synthesizer, operating in a 0.12 mmol scale on a HMPB-ChemMatrix resin (0,45
46 mmol/g). Resin was swelled prior to use with a NMP/DCM mixture. Activation and coupling of
47 Fmoc-protected amino acids was performed using Oxyma 0.5M / DIC 0.5M (1:1:1), with a 5
48 equivalent excess over the initial resin loading. Coupling steps were performed for 7 minutes at

75°C. Deprotection steps were performed by treatment with a 20% piperidine solution in DMF at room temperature (1 x 3 min + 1 x 5min). Following each coupling or deprotection step, peptidyl-resin was washed with DMF (4 x 5ml). Following chain assembly, peptide was cleaved from the resin using a TFA 90%, water 5%, thioanisole 2.5%, TIS 2.5% mixture (3 hours, RT). Following precipitation in cold diethyl ether, crude peptide was collected by centrifugation and washed with further cold diethyl ether to remove scavengers. Peptides was then dissolved in 50% aqueous acetonitrile 0.07% TFA buffer and purified by preparative RP-HPLC.

RP-HPLC analysis and purification

Analytical and semi-preparative reversed phase high performance liquid chromatography (RP-HPLC) were carried out on a Tri Rotar-VI HPLC system equipped with a MD-910 multichannel detector for analytical purposes or with a Uvidec-100-VI variable UV detector for preparative purpose (all from JASCO, Tokyo, Japan). A Phenomenex Jupiter 5 μ C18 90Å column (150 x 4.6 mm) was used for analytical runs and a Phenomenex Jupiter 10 μ C18 90Å (250 x 21.2 mm) for peptide purification. Data were recorded and processed with Borwin software. 2 % /min linear gradient of 0-60 % eluent B (eluent A = H₂O/ 3 % CH₃CN / 0.07 % TFA, eluent B = 70 % CH₃CN/ 30 % H₂O/ 0.07 % TFA) was employed at a flow rate of 1mL/min for analytic purposes. UV detection was recorded in the 220-320 nm range. Peptide purification was achieved by preparative RP-HPLC at a flow rate of 14 mL/min using a 100% A $\rightarrow$ 30% B gradient over 40 min. Pure RP-HPLC fractions (>95%) were combined and lyophilized.

Reagents

HMPB-ChemMatrix resin and *N*-a-Fmoc-L-amino acids used during chain assembly were purchased from Iris Biotech GmbH (Marktredwitz, Germany). Ethyl cyanoglyoxylate-2-oxime (Oxyrna) was purchased from Novabiochem (Darmstadt, Germany), *N,N'*-dimethylformamide (DMF) and trifluoroacetic acid (TFA) were from Carlo Erba (Rodano, Italy). *N,N'*-diisopropylcarbodiimide (DIC), dichloromethane (DCM) and all other organic reagents and

74 solvents, unless stated otherwise, were purchased in high purity from Sigma-Aldrich (Steinheim,
75 Germany). All solvents for solid-phase peptide synthesis (SPPS) were used without further
76 purification. HPLC grade acetonitrile (ACN) and ultrapure 18.2 Ω water (Millipore-MilliQ) were
77 used for the preparation of all buffers for liquid chromatography. The chromatographic columns
78 were from Phenomenex (Torrance CA, USA)

79

80 **Flow cytometry**

81 Cells were stained with monoclonal anti-human fluorescently conjugated antibodies: CD14
82 (M5E2), CD19 (HIB19), CD11c (B-ly6), CD45RA (HI100), CD3 (UCHT1), TNF (Mab11), IFN-
83 (4S.B3), TNF (Mab11) from BD Biosciences; CD4 (RPA-T4) from Ebioscience; CD1a (HI149),
84 HLA-DR (L243), CD86 (IT2.2) and TSLPR (1B4) from Biolegend. Myeloid cells isolated from
85 PBMC were stimulated in complete RPMI 1640 (10^6 cells/condition) with an equimolar dose of
86 short TSLP and/or long TSLP (3,5 nM) for 15 minutes at 37°C. Control conditions included cells
87 either left unstimulated (negative control) or incubated with sodium pervanadate (Na_3VO_8 , positive
88 control, data not shown). At the end of incubation cells were rapidly spun, fixed with BD Cytotfix™
89 Buffer for 15 minutes at 37°C and permeabilized with BD™ Phosflow Perm Buffer III on ice for 30
90 minutes, followed by intracellular staining using mouse anti-pSTAT5 (pY694) antibody (45/Stat5,
91 BD Biosciences) or isotype antibody (MOPC-21). Samples were acquired on a BD CantoII flow
92 cytometer (BD Biosciences) and analyzed with FlowJo software (v8.7, Tree Star).

93

94 **Monocyte-derived dendritic cell differentiation and stimulation conditions**

95 Peripheral CD14⁺ monocytes were isolated from PBMC using human CD14⁺ microbeads (Miltenyi
96 Biotec) according to the manufacturer's instructions. Cells were then cultivated in complete RPMI
97 1640 medium in the presence of IL4 (2,5 ng/ml) and GM-CSF (5 ng/ml) (BD Biosciences) for 6

98 days to obtain monocyte-derived dendritic cells. The percentage and the phenotype of cell subsets
99 were evaluated before and after differentiation by flow cytometry. Dendritic cells were seeded in
100 complete RPMI 1640 medium at 1×10^6 /ml in the presence of short TSLP or culture medium alone.
101 After 24 hours of culture, dendritic cells were gently washed and challenged with *Salmonella*
102 thyphimurium FB62 (MOI 1:1) for 1 hour. Bacteria were then washed out and the medium replaced
103 with medium containing 100 µg/ml gentamycin. Cytokine secretion was assessed after 24 hours by
104 cytometric bead assay Flex sets (BD Biosciences). CBA beads were acquired on a BD Accuri C6
105 (BD Biosciences) and analyzed with FCAP software (BD Biosciences). For signaling experiments
106 fully differentiated dendritic cells were left untreated or stimulated with short TSLP (100 ng/ml, 5
107 min and 20 min), LPS (100 ng/ml, 10 min) or with short TSLP (24 hours) and LPS (10 min). Cells
108 were then lysed for western blot analysis or for Human Phospho-Kinase Array (R&D Systems,
109 ARY003B) analysis. The Human Phospho-Kinase Array analysis was performed following
110 manufacturer's instructions; quantification analysis was performed using ImageJ software.

111

112 **Myeloid dendritic cell isolation and stimulation conditions**

113 Myeloid BDCA1⁺ cells were isolated from PBMC using CD1c (BDCA-1)⁺ Dendritic Cell Isolation
114 Kit (Miltenyi Biotec) according to the manufacturer's instructions. Cells were then cultivated in
115 complete RPMI medium in the presence of long TSLP or short TSLP at the indicated doses, for 24
116 hours. Cells were then washed and co-cultured with naïve CD4⁺ T cells from a different donor
117 (ratio 1 DC: 5 T). CD4⁺ cells were isolated using naïve CD4⁺ T Cell Isolation Kit II (Miltenyi
118 Biotec). After 6 days, supernatants were collected and cells were restimulated for 4 hours with
119 PMA (50 ng/ml) and ionomycin (1 µg/ml); for the last 3 hours brefeldin A (10 µg/ml) was added to
120 the culture. Intracellular staining for TNF- and IFN- positive cells was performed afterwards.
121 CCL17, CCL22 and IFN- levels were assessed in the supernatant of DC or of the DC-T culture by
122 ELISA (R&D Systems).

123

124 **Epithelial cells and keratinocytes culture**

125 Caco2 and HaCaT cells were cultured in DMEM (Lonza) supplemented with 10% FBS, 1%
126 glutamine, 1% non essential aminoacids, 1% penicillin-streptomycin. HEKa keratinocytes (Gibco)
127 were cultured in EpiLife® medium supplemented with HKGS growth factor (Gibco) and 1%
128 penicillin-streptomycin. Confluent Caco2 cells were infected with bacteria in the absence of
129 antibiotics at a multiplicity of infection of 1:100 for 1 hour. Then medium was replaced with
130 complete medium containing 100 µg/mL gentamicin and the cells were cultured for a further 23
131 hours. Confluent HEKa and HaCaT cells were challenged with calcitriol, an active form of vitamin
132 D (10 µM, Enzo Life Sciences), or poly I:C (1 or 5 µg/mL) (Invivogen) for 8 or 24 hours. HEKa
133 cells were starved for 24 hours before stimulation. At the end of the challenge cells were lysed in
134 Trizol reagent for RNA extraction, fixed in 1% formaldehyde for ChIP analysis or RIPA buffer for
135 protein extraction. Figures show results from three independent experiments for Caco2 and HaCaT
136 cells. Protein and mRNA expression are expressed as fold change compared with untreated cells
137 (a.u.=1).

138

139 **Long TSLP specific antibody production**

140 For anti-long TSLP specific antibody preparation, the fragment of the long isoform absent in the
141 short (fragment TSLP, aa 1-96) was cloned and expressed in BL21 competent *E.coli* cells with a
142 DsbA tag. Rabbits were immunized with the tagged fragment in the presence of incomplete
143 Freund's adjuvant and the resulting sera were depleted of anti-DsbA antibodies. The final
144 preparation has been tested for its specificity in Western Blot against the two different purified
145 isoforms and in comparison with commercially available antibodies recognizing both isoforms
146 (Abcam).

147

148 **Immunofluorescence**

149 Healthy and IBD mucosa was fixed in Hollande's fixative (Polysciences Inc.) and paraffin-
150 embedded in a Leica ASP300 tissue processor. 5 μ m-thick sections were deparaffinized and
151 rehydrated. Antigen unmasking (Vector laboratories solution) was performed at 95°C for 50
152 minutes. AD biopsies were included in OCT. 5 μ m-thick sections were thawed for 5 minutes at
153 room temperature and fixed for 15 minutes in 4% PFA. Sections were washed 3 times with TBS.
154 All sections were blocked in TBS-Tween 0.05% containing normal donkey serum. Primary
155 antibodies were incubated overnight at 4°C: anti- total TSLP (Abcam), anti-long TSLP (in-house)
156 and anti-CD14 (Abnova) antibodies. Secondary antibodies, donkey anti-rabbit 555 and donkey anti-
157 mouse 488, Molecular Probes, were incubated for 1 hour and sections were counterstained with
158 DAPI. Slides were mounted with Vectashield (Vector laboratories) and visualized under a Leica
159 SP2 confocal microscope (40X magnification). Figures shown are representative of 5 different
160 patients or controls.

161

162 **Protein extraction and Western blot**

163 Protein concentration of cell lysates were determined by Bradford assay (Biorad). Lysates (30 μ g)
164 were then separated by Tris-Tricine SDS-PAGE gels (16% of acrylamide) and electrophoretically
165 transferred to a PVDF membrane (Millipore). For immunoblots, membranes were incubated
166 overnight at 4°C with mouse anti-vinculin antibody, rabbit anti-human TSLP antibody (Abcam),
167 rabbit anti-human long TSLP antibody generated in house, rabbit anti-p38 MAPK (Cell Signaling)
168 and rabbit anti-phospho-p38 MAPK (Cell Signaling). Protein levels are normalized to vinculin
169 expression. Western blot quantification analysis was performed using ImageJ software.

170

171 **Chromatin Immunoprecipitation**

172 ChIP experiments were performed with HEK293 cells using $3-5 \times 10^6$ for condition. Cells were fixed at
173 room temperature for 10 minutes at room temperature with 1% formaldehyde and quenched with
174 50mM Tris-HCl pH7.6 for 5 minutes. Subsequently cells were lysed for 10 min at 4°C in LB1
175 buffer (50mM HEPES, pH 7.5, 140mM NaCl, 1mM EDTA, 10% glycerol, 0.25%Triton, 0.5%
176 NP40, 1mM PMSF, 10ug/ml aprotinin, 10ug/ ml leupeptin), collected by centrifugation and washed
177 in LB2 buffer (10mM Tris-HCl pH 8, 200mM NaCl, 1mM EDTA, 0.5% EGTA, PMSF, aprotinin,
178 leupeptin) for additional 10 minutes at room temperature. Nuclei were collected by centrifugation
179 and resuspended in LB3 buffer (10mM Tris-HCl pH 8, 100mM NaCl, 1mM EDTA, 0.5mM EGTA,
180 0.1% Na-Deoxycholate, 0.5% N-lauroylsarcosine, 1mM PMSF, 10ug/ml aprotinin, 10ug/ml
181 leupeptin) for sonication (Bioruptor, 50 pulses of 30sec each, followed by 30sec of resting in a
182 refrigerated water bath). A fraction of material was saved as “Input”. The nuclear extracts were then
183 incubated on a rotating wheel over night at 4°C with Dynabeads Protein G (Life technologies)
184 previously coated with 3 ug of Anti-Histone H3 (acetyl K27) antibody (Abcam) or same amount of
185 normal rabbit IgG. The beads were washed five times with RIPA buffer and immunocomplexes
186 eluted at 65°C for 15 min with TE-2%SDS. Eluates were reverse cross-linked by heating at 65°C
187 over night and DNA was purified with QIAquick PCR purification kit (Qiagen).
188 The analysis of immunoprecipitated DNA sequences was performed through real-time PCR with
189 Fast SYBR Green PCR kit on the Applied Biosystems 7900HT Fast RT-PCR System (Applied
190 Biosystems) and results presented as percentage of the input DNA. The primers for long and short
191 TSLP as well as primers for positive and negative regions used as controls are listed in Repository
192 Table E1.

193

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195

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205

206

207

208 **Figure legends**

209

210 **Fig. E1: Available forms of long and short TSLP.** Western blot of commercially available long
211 TSLP (long E.coli, R&D systems), in-house produced long TSLP (long Baculovirus) and in-house
212 synthesized short TSLP peptide (short peptide). The membrane is blotted with two antibodies, one
213 in-house made recognizing only the long TSLP isoform (left) and one commercially available and
214 recognizing both isoforms (right, Abcam).

215

216 **Fig. E2: ShTSLP did not interfere with the maturation of DCs.** Flow cytometry analysis of
217 CD86 and HLA-DR expression on moDCs, conditioned or not, with shTSLP for 24 hours and
218 challenged with *Salmonella* (MOI 1:1) for 1 hour.

219

220 **Fig. E3: Short TSLP conditioned moDC show decreased phosphorylation of p38 and ERK1/2**
221 **after LPS challenge.** Human Phospho-Kinase array analysis on moDC left untreated, incubated 10
222 minutes with 100 ng/ml LPS (LPS) or conditioned 24 hours with short TSLP (100ng/ml) and then
223 challenged with LPS (shTSLP + LPS).

224

225 **Fig. E4: Sequences of human long TSLP and murine TSLP.** Sequence alignment of human
226 lTLSP and murine TSLP with secondary structure annotation based on the crystallographic
227 structure. Shaded residues are identical residues between mouse and human TSLP.

228

229 **Fig. E5: TSLPR expression in gut and skin biopsies.** qPCR analysis of TSLPR in intestinal cells
230 from healthy individuals (H), ulcerative colitis (UC), untreated (UCD) and treated (TCD) coeliac
231 patients (A,B); in skin biopsies of lesional (L) or non-lesional (NL) atopic dermatitis patients (C).

232

233 **Fig. E6: Long TSLP protein production is increased in ulcerative colitis compared to healthy**
234 **tissues.** Western blot analysis (upper panel) and quantification (bottom panel) of long and short
235 TSLP expression in tissue biopsies from healthy individuals (n=9) and ulcerative colitis patients
236 (n=9). Values are normalized to vinculin expression. For short TSLP quantification all
237 degradation/cleavage fragments have been considered.

238

239 **Fig. E7: shTSLP is the only isoform expressed at steady state in epithelial cells.** Expression of
240 short and long TSLP in untreated Caco2 cells by real-time PCR (A) and by western blot (B)
241 analysis. The 'fold induction' in mRNA expression is in reference to that of the 'housekeeping'
242 gene, *gapdh*. Protein levels, detected with antibodies against long TSLP isoform and total TSLP
243 isoforms, are normalized to vinculin expression.

244

245 **Fig. E8: TSLP isoforms are differentially regulated in human keratinocytes.** qPCR analysis of
246 TSLP isoforms in untreated (A) or stimulated (B) with vitamin D3 (10 μ M) and poly I:C (1 μ g/ml)
247 HaCaT cells for 24 hours. Results (B) are represented as fold increase compared to untreated cells.

Table E1. Primer list

RT-PCR

Gene	Forward (5'-3')	Reverse (5'-3')
<i>gapdh</i>	ATCAGCAATGCCTCCTGCAC	TGGCATGGACTGTGGTCATG
<i>krt18</i>	TGATGACACCAATATCACACGAC	TACCTCCACGGTCAACCCA
<i>tslp</i> (long isoform)	CACCGTCTCTTGTAAGCAATCG	TAGCCTGGGCACCAGATAGC
<i>tslp</i> (short isoform)	CCGCCTATGAGCAGCCAC	CCTGAGTAGCATTATCTGAG
<i>crlf2</i> (TSLPR)	AGAGCAGCGAGACGACATTC	CCGGTACTGAACCTCATAGAGG

ChIP qPCR

Gene	Forward (5'-3')	Reverse (5'-3')
<i>gapdh</i>	TACTAGCGGTTTTACGGGCG	TCGAACAGGAGGAGCAGAGAGCGA
<i>tslp</i> (long isoform)	CCTAGGAGAAAAGAGCCCGT	GCTGTTGCCTTAAGTTGGAGT
<i>tslp</i> (short isoform)	AAGGCAAATTGGGAAGTGGG	GAGGCTGCCAAAATTCCGAG
negative control	TTCTCCATTCTCCCTCACCT	TTGTGGGGAAAGGAGTTTG

Table E2. Patient list

Ulcerative colitis (UC) n=13				
Age (mean $\pm$ SD, years)			41 $\pm$ 14	
Sex (male/female)			9/4	
Patient	Age (y)	Colon tract	Sex	Treatment
1	31	sigma	F	5ASA, Deltacortene 45 mg per os, Iron 1 cps
2	44	sigma	F	5ASA per os
3	54	sigma	F	6MP, deltacortene 15 mg, Neolotan
4	21	sigma	F	Deltacortene 15 mg, Lexil, Prefolic, Cacit D3
5	53	sigma	M	Deltacortene 5 mg, Aprovel, Congescor, Clexane, Kanrenol
6	34	sigma	M	Deltacortene 40 mg, 5ASA per os, Pariet 20 mg, Calcium, Dibase
7	66	sigma	M	Topster suppository, Inderal tablets
8	60	sigma	M	none
9	29	sigma	M	5ASA per os, Pariet
10	31	rectus	M	Ramipril, Bisoprololo, Ferrograd
11	37	sigma	M	steroid per os, 5ASA per os
12	33	sigma	M	Deltacortene 20 mg, Cacit D3, Pariet 20 mg, Insulin R
13	38	sigma	M	Deltacortene 10 mg

Healthy tissue from CRC patients (H) n=26	
Age (mean $\pm$ SD, years)	72 $\pm$ 12
Sex (male/female)	16/9
Tract (colon/ileum)	22/3

Untreated coeliac disease (UCD) n=13	
Age (mean $\pm$ SD, years)	43 $\pm$ 14
Sex (male/female)	7/6
Histological grading (B1/B2) ^{E4}	7/6

Treated coeliac disease (TCD) n=15	
Age (mean $\pm$ SD, years)	40 $\pm$ 15
Sex (male/female)	6/9
Histological grading ^{E4}	A

Healthy tissue (H) n=13	
Age (mean $\pm$ SD, years)	44 $\pm$ 12
Sex (male/female)	8/5

Atopic dermatitis (AD) n=24											
Age (mean ± SD, years)				46±17							
Sex (male/female)				16/8							
Patient	Age	Sex	PAS I	PSI	SCORA D	Allergies	Prick	IgE	Atopic diseases	Family history PV	Family history atopic diseases
1	47	F	n.a.	n.a.	46,5	nickel, birch, ampicillin	gras, birch polls, Dermatopahgoides pter., cat	raised	asthma	negative	positive (daughter: allergic rhinitis, asthma)
2	38	F	n.a.	n.a.	75	cat	cat	raised	negative	negative	negative
3	38	F	n.a.	n.a.	63	horse, grass + birch pollen, hazelnut, fruits	grass + birch pollen	raised	allergic rhinitis, allergic conjunctivitis	negative	parents: AR; Brother AD + Asthma
4	47	F	n.a.	n.a.	38,5	horse, cat, dog, grass + birch pollen, Dermatopahgoide s pter + far	cat, dog, grass + birch pollen, Dermatopahgoides pter	raised	allergic rhinitis, allergic conjunctivitis, asthma	negative	positive (daughter: allergic rhinitis)
5	38	F	n.a.	n.a.	41	colophonium, thiuram	n.d.	n.d.	negative	negative	negative
6	47	F	n.a.	n.a.	61	negative	negative	n.d.	negative	negative	negative
7	22	F	n.a.	n.a.	85,5	peanuts, grass polls, derm. Pter + far., cat	grass + birch polls, derm. Pter, cat	raised	allergic rhinitis, allergic conjunctivitis, asthma	negative	negative
8	82	F	n.a.	n.a.	63,5	grass + birch pollen	grass pollen	raised	allergic rhinitis, allergic conjunctivitis, asthma	negative	negative
9	55	M	n.a.	n.a.	59	none	n.d.	n.d.	negative	negative	negative
10	45	M	n.a.	n.a.	52	fish, grass pollen, Dermatopahgoide s pter + far	cat, grass pollen, Dermatopahgoides pter + far	n.d.	negative	negative	positive (son: AD)
11	43	M	n.a.	n.a.	66	pollen, drugs	n.d.	n.d.	allergic rhinitis, allergic conjunctivitis	negative	positive (brother: AR)
12	19	M	n.a.	n.a.	45	not known	n.d.	n.d.	allergic rhinitis, allergic conjunctivitis	negative	positive (sister: AR, brother: allergic

											rhinitis)
13	67	M	n.a.	n.a.	89	Nickel, grass pollen, Dermatopahgoides pter + far	Dermatopahgoides pter + far	no	allergic rhinitis, allergic conjunctivitis	negative	positive (father + son: AD)
14	48	M	n.a.	n.a.	55	negative	negative	raised	none	negative	positive (father + daughter: AD)
15	25	M	n.a.	n.a.	82,5	food, grasspolls, dust mites, dog, cat	grass + birch pollen, Dermatopahgoides pter + far, cat, dog	> 5000IU/ml	allergic rhinitis, allergic conjunctivitis, asthma	negative	positive (mother: AD, asthma; Brother: AD)
16	47	M	n.a.	n.a.	57	birch + grass polls, dust mites, dog, mugword, nickel	grass, Dermatopahgoides pter, dog	152IU/ml	allergic rhinitis, allergic conjunctivitis	negative	positive (brother: AR, AC, AD)
17	19	M	n.a.	n.a.	75	birch polls	grass, cat, Dermatopahgoides pter	raised	negative	negative	positive (mother: AD, asthma)
18	23	M	n.a.	n.a.	64,5	grass polls, dust mites, dog, cat	grass, cat, dog, Dermatopahgoides pter	1450 IU/ml	n.a.	n.a.	negative
19	45	M	n.a.	n.a.	78,5	none	n.d.	n.d.	negative	negative	mother: AD, Allergic rhinitis, Allergic conjunctivitis, asthma, urticaria
20	59	M	n.a.	n.a.	73	none	n.d.	> 400 IU/ml	negative	negative	negative
21	65	M	n.a.	n.a.	57,5	dust mites, cat, dog	Dermatopahgoides pter	> 400 IU/ml	Allergic rhinitis, Allergic conjunctivitis	negative	unkonown (mother: AD ?)
22	50	M	n.a.	n.a.	43,5	none	n.d.	n.d.	allergic rhinitis	negative	negative
23	74	M	n.a.	n.a.	37	none	n.d.	raised	n.a.	n.a.	n.a.
24	69	M	n.a.	n.a.	56,5	none	negative	raised	negative	negative	son: AD, allergic rhinitis, asthma; brother: asthma

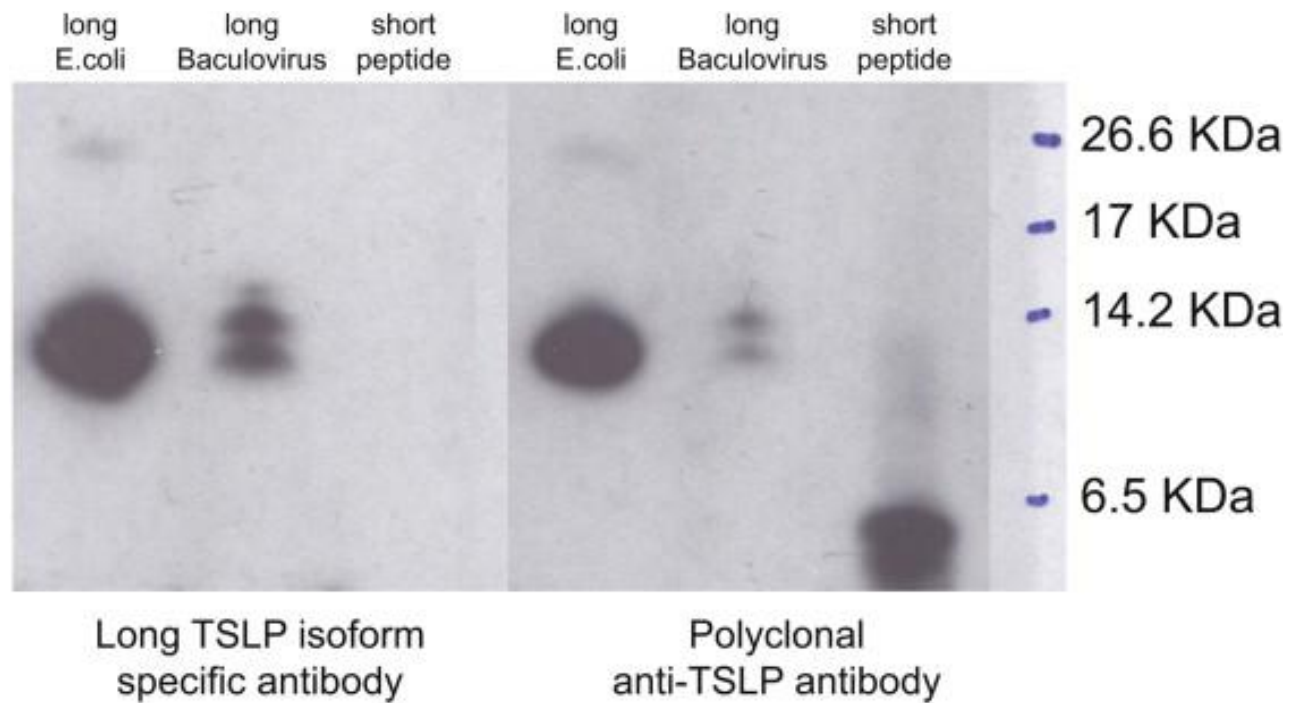


Fig E1

Giulia Fornasa , Katerina Tsilingiri , Flavio Caprioli , Fiorenzo Botti , Marina Mapelli , Stephan Meller , Andrea...

Dichotomy of short and long thymic stromal lymphopoietin isoforms in inflammatory disorders of the bowel and skin

Journal of Allergy and Clinical Immunology, 2015

<http://dx.doi.org/10.1016/j.jaci.2015.04.011>

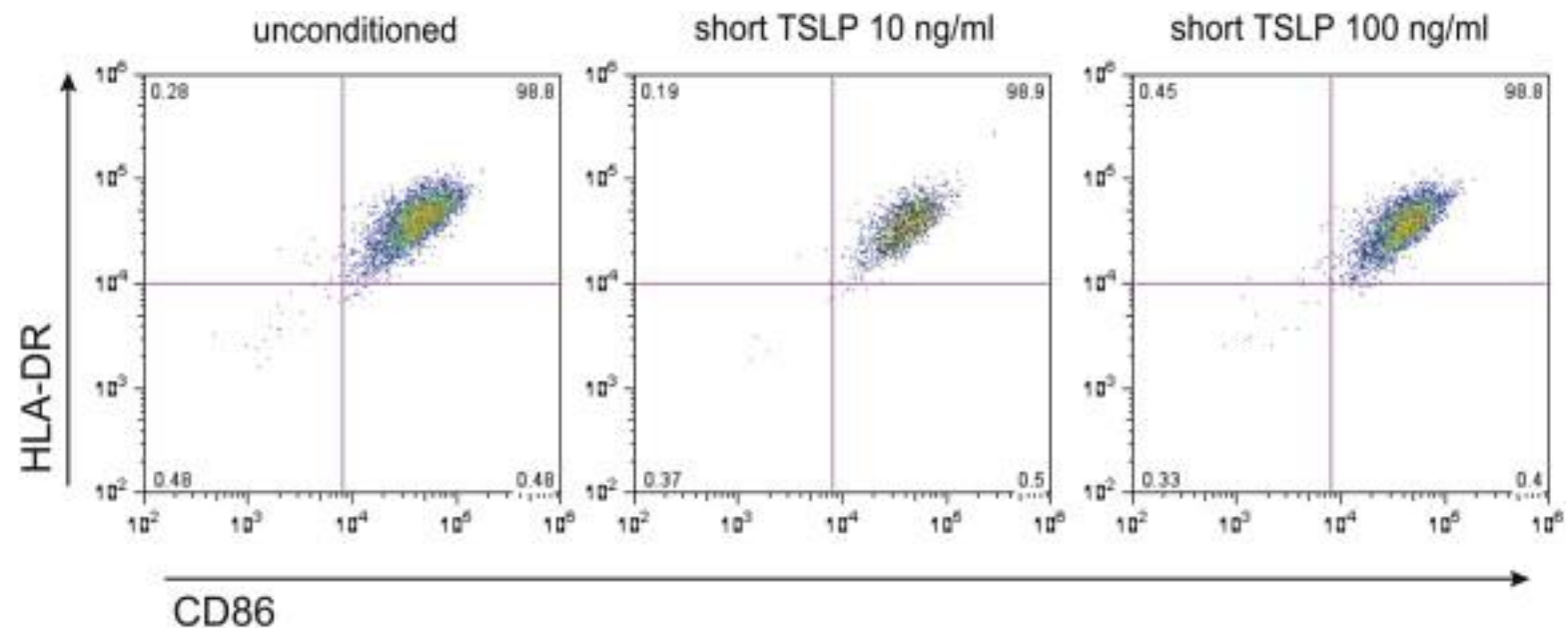


Fig E2

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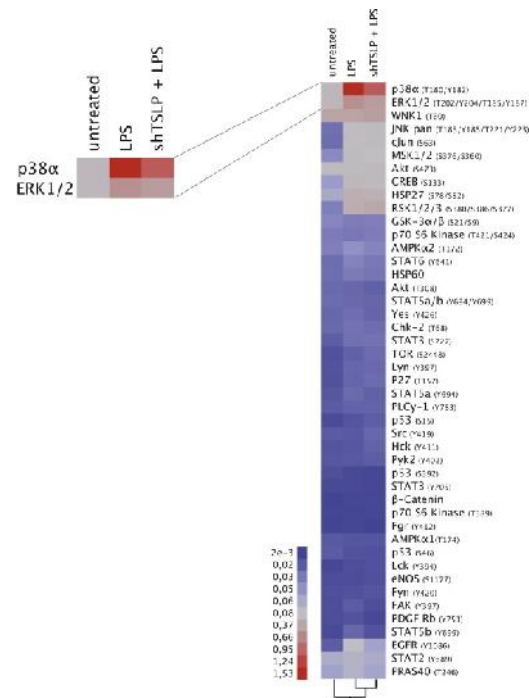


Fig E3

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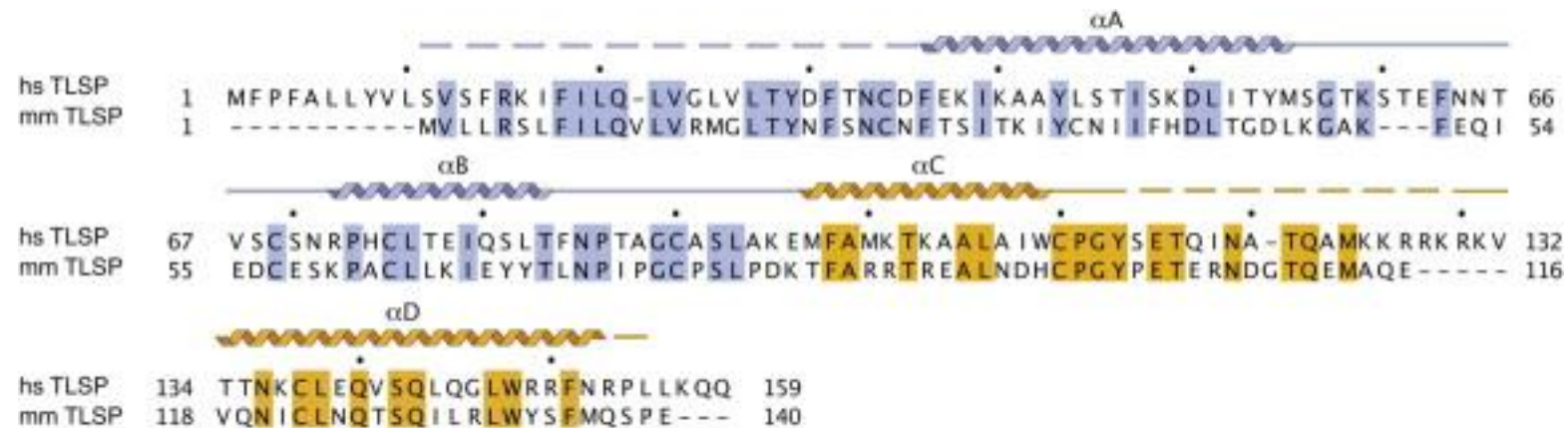


Fig E4

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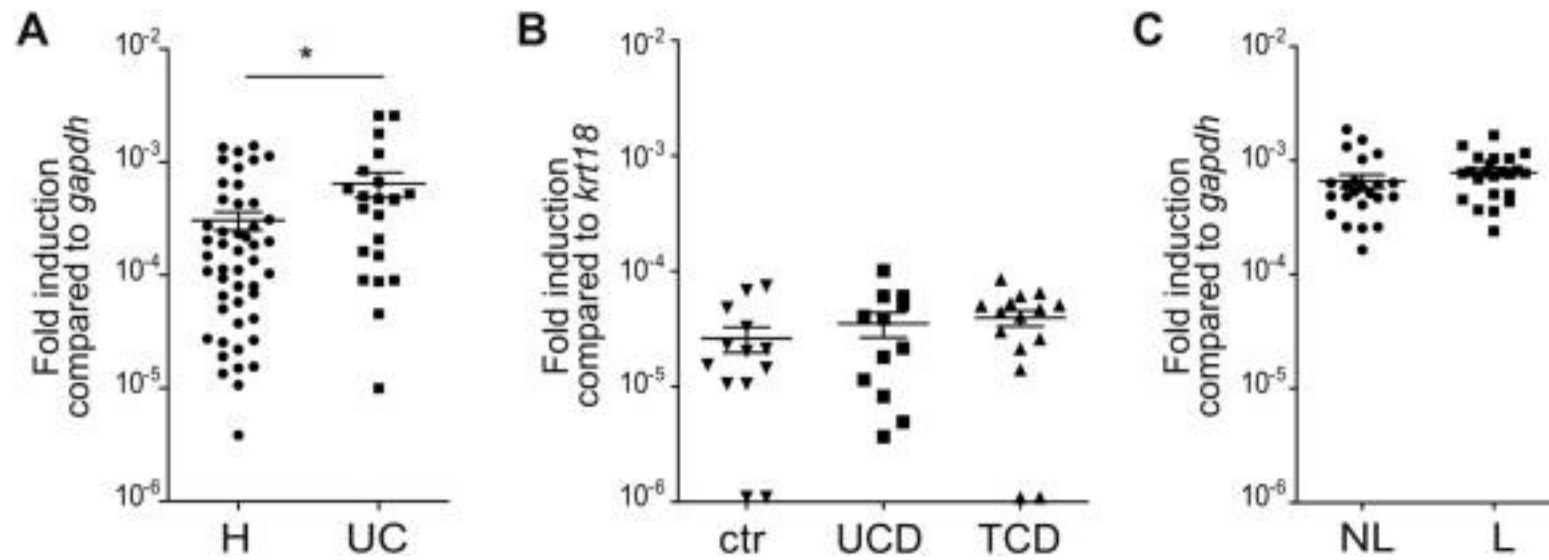


Fig E5

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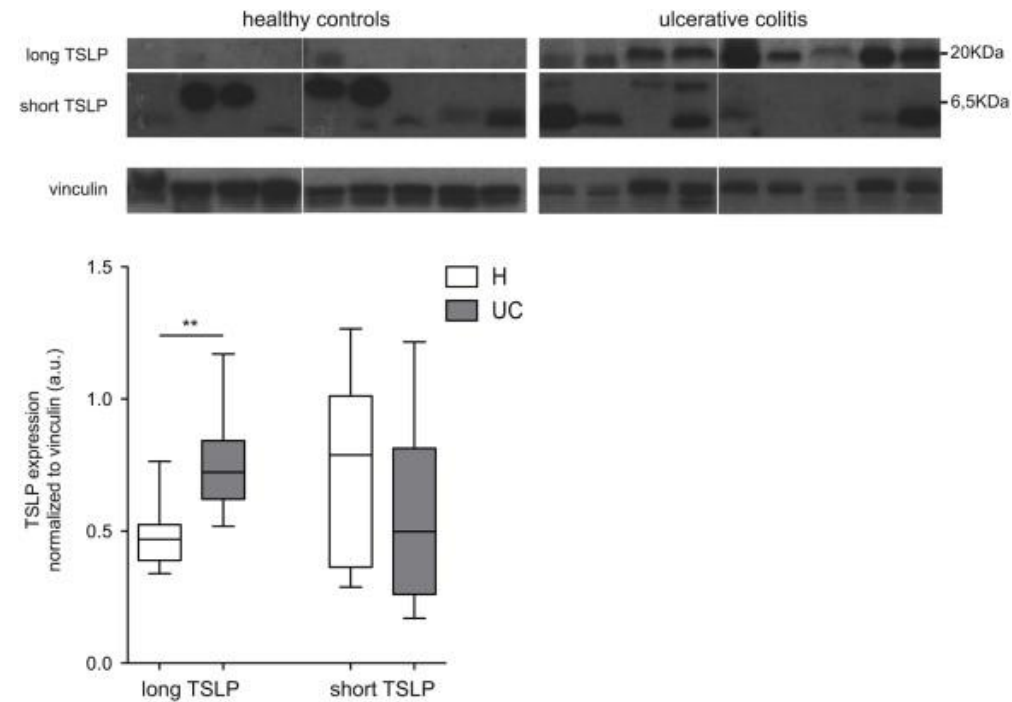


Fig E6

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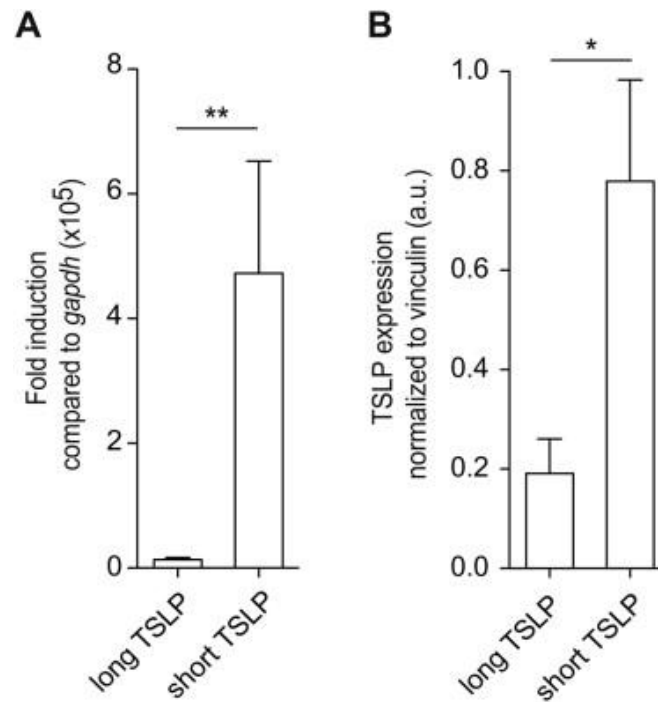


Fig E7

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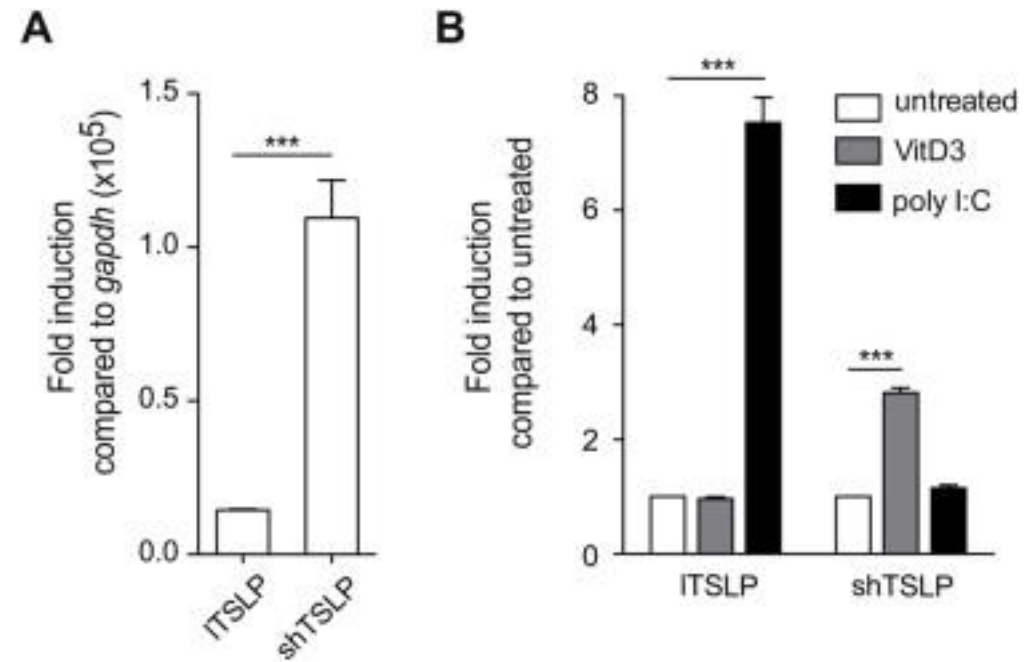


Fig E8

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