Supporting Information

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SI Materials and Methods

**Reagents.** SP, IL-1, and L-703,606 were purchased from Sigma. IL-33 was purchased from Antigenex America. PD98059, SP600125, SB203580, Bisindolylmaleimide I, and Go6976 were purchased from Calbiochem. Cortistatin-17 was purchased from Phoenix Pharmaceuticals. All MAPK antibodies were purchased from Cell Signaling Technology. L-733,060 was purchased from Sigma-Aldrich.

**Culture of Human Mast Cells (hCBMCs).** These cells were cultured in Iscove’s Modified Dulbecco’s Medium (IMDM; Gibco), containing 200 ng/mL recombinant human stem cell factor (rhSCF) from Amgen and 50 ng/mL IL-6 (Millipore) for 12–16 weeks. IL-4 (20 ng/mL) was used for 2 weeks before the experiment. All cells were maintained at 37 °C in a humidified incubator at 5% CO2.

**Cytosolic Calcium Measurements.** Cytosolic calcium was measured in LAD2 cells at 37 °C using Fura-2 as indicator. LAD2 cells were loaded in Tyrode’s buffer with 1 mM Fura-2 AM (Invitrogen) for 20 min to allow Fura-2 to enter the cells. After centrifugation to remove excess dye, the cells were resuspended in Tyrode’s buffer at a concentration of 1 million cells/mL and incubated for another 20 min. Cells were then transferred to 96-well plates with 100 μL per well. SP (1 μM) was added to cells for the time indicated. Fura-2 fluorescence was read by MDC FlexStation II (Molecular Devices) at an excitation wavelength of 340 nm/380 nm and emission wavelength of 510 nm. Results were processed according to the Invitrogen Fura-2 protocol.

**VEGF Release Assay.** Mast cells (1 × 10^5 cells/200 μL) were distributed in 96-well microtiter assay plates in triplicates and stimulated in complete culture medium with the indicated concentrations of SP. For inhibition studies, inhibitors were added to the media 30 min before stimulation. VEGF was determined in cell-free supernatants with a commercial ELISA kit (R&D Systems) according to the manufacturer’s directions. VEGF secretion data are expressed as pg/10^6 cells.

**PCR and Quantitative PCR.** Total RNA from skin biopsy samples or cultured mast cells was isolated using TRIzol (Invitrogen), according to the manufacturer’s instructions. Reverse transcription was performed with 200 ng of total RNA using the iScript cDNA synthesis kit (Ambion). PCR was performed with GoTaq Green master mix (Bio-Rad) and 400 nM of the following primers; β-actin forward, 5′-TGGTATGTTGGAGATGGTCAG-3′ and β-actin reverse, 5′-TCTTGATGTCACCGACGATTTCC-3′, which amplifies a 511-bp fragment. The cycling conditions consisted of 35 cycles of 95 °C for 15 s and 60 °C for 1 min.

Quantitative PCR was performed in triplicate with an Applied Biosystems 7300 Real-Time PCR System using iTaq SYBR Green Supermix (Bio-Rad) and 400 nM of each primer. The following probes, obtained from Applied Biosystems, were used: IL-33 (Assay ID: Hs00369211_m1); HDC (Assay ID: Hs00157914_m1), and TAC1 (Assay ID: Hs00243227_m1). Human GAPD (GAPDH) Endogenous Control (VIC/TAMRA Probe, Primer Limited; Part Number: 4310884E). The cycling conditions consisted of 1 cycle of 50 °C for 2 min, 1 cycle of 95 °C for 10 min, 40 cycles of 95 °C for 15 s, 1 cycle of 60 °C for 1 min, 1 cycle of 95 °C for 15 s, 1 cycle of 60 °C of 30 s, and 1 cycle of 95 °C for 15 s. Relative mRNA abundance was determined from standard curves run with each experiment, and IL-33, HDC and TAC1 expression was normalized to GAPDH Endogenous Control.

**Patients and Biopsies.** All skin biopsies requiring two stitches were performed in subjects (patients and controls) who had not received any medication for 15 days before the biopsy and were seen at the second Department of Dermatology of the Attikon General Hospital, Athens University Medical School (Athens, Greece). The Medical Ethics Committee of Attikon Hospital Institution’s Human Investigation Review Board approved this protocol. All participants gave their written informed consent according to the Declaration of Helsinki principles. Patients had moderate chronic plaque psoriasis with Psoriasis Area and Severity Index (PASI) scores of 3–16. Patients were free of any other medical problems. All biopsy samples, from both patients and controls, were obtained from nonexposed skin (back and gluteal). Samples of unaffected skin of psoriasis patients were obtained from sites at least 15 cm away from the affected lesional areas. All biopsy samples were immediately placed in RNAlater solution (Ambion) and stored at −20 °C.

**Western Blot Analysis of MAPK Phosphorylation.** LAD2 cells were plated in 24-well plates (10^5 cells/well) in complete media. Cells were stimulated with SP (1 μM) for the indicated time points. Stimulation was terminated by the addition of ice-cold PBS. Cells were washed once with PBS and then lysed in buffer containing 20 mM Tris-HCl, pH 7.4, 1 mM EDTA, 150 mM NaCl, 1% Nonidet P-40, 0.1% SDS, 1 mM PMSF, 10 μg/mL aprotinin, 10 μg/mL leupeptin, 1 mM NaVO4, and 50 mM NaF. Equal amounts of protein were electrophoresed on 4–12% polyacrylamide gels (Invitrogen) and then transferred to a 0.44-μm PVDF membrane (Invitrogen). After blocking with 5% BSA, membranes were probed with antibodies against the phosphorylated form of ERK1/2 (1:2,000 dilution), JNK, or p38 (1:1,000 dilution). For detection, the membranes were incubated with the appropriate secondary antibody conjugated to HRP (1:2,000 dilution) and the blots were visualized with enhanced chemiluminescence. Blots were then stripped and reprobed with antibodies against total ERK, JNK, or p38 at 1:1,000 dilution.

**IL-33 Immunohistochemistry.** Skin biopsy samples from patients with psoriasis and control subjects were immediately placed in Tissue Freezing Medium (TBS, catalog no. H-TFM) and stored at −80 °C. Five sections (5 μm) were cut using a cryostat and fixed with cold acetone for 10 min. Immunohistochemical staining was performed using the LSAB+ system kit (DAKO). Incubation with the primary antibody (mouse monoclonal anti-human–IL-33 antibody, at 1:100 dilution; Abcam) was performed for 30 min; secondary antibody was provided in the DAKO kit and was also used for 30 min, followed by appropriate washes. As negative control, the primary antibody was omitted. Faramount, Aqueous Mounting Medium (DAKO) was used for aqueous mounting. Sections were examined by three different investigators and photographs were taken using an upright Olympus BH2 microscope.

Theoharides et al. www.pnas.org/cgi/content/short/1000803107
Fig. S1. PKC inhibitors partially inhibit SP-induced VEGF release from LAD2 cells. LAD2 cells were preincubated for 30 min with the indicated concentrations of the nonselective PKC inhibitor, bisindolylmaleimide I (Bis) (A) G06976 (B). Cells were stimulated with SP (1 μM) for 24 h and supernatant VEGF was measured by ELISA. Data are mean ± SD of three separate experiments performed in triplicates (*P < 0.05 vs. SP-stimulated cells).

Fig. S2. SP induces ERK and JNK phosphorylation in LAD2 cells. Cells were stimulated with SP (1 μM) for up to 120 min. Cell lysates were subjected to 4–12% SDS/PAGE and immunoblotted with (A) phospho-ERK or (B) phospho-JNK–specific antibodies. Membranes were stripped and reprobed with the respective total MAPK antibody. Results are representative of three separate experiments.

Fig. S3. MAPK inhibitors block SP-induced VEGF release from LAD2 cells. LAD2 cells were preincubated for 30 min with the indicated concentration of (A) upstream ERK inhibitor PD98059 (ERKinhibitor) and (B) JNK inhibitor SP60012 (JNKinhibitor). Cells were stimulated with SP (1 μM) for 24 h and supernatant VEGF was measured by ELISA. Data are mean ± SD of three separate experiments performed in triplicate. *P < 0.05 vs. unstimulated cells (#P < 0.05 vs. SP-stimulated cells).
Fig. S4. Diagrammatic representation of the proposed interrelationships in the unaffected and affected skin from psoriasis patients. SP synthesized in a neuron in the unaffected skin is transported and released from terminals in the affected psoriatic skin. Increased gene expression of IL-33 in the unaffected areas can derive from endothelial, epithelial cells, and/or from increased number of mast cells as evidenced by elevated HDC expression. In the affected skin, increased IL-33, possibly coming from infiltrating lymphocytes, proliferating keratinocytes, and endothelial cells, can augment SP-induced mast cell release of VEGF, leading to increased vascular permeability and inflammation.