IL-33 augments substance P–induced VEGF secretion from human mast cells and is increased in psoriatic skin

Theocharis C. Theocharides1,2,4, Bodi Zhang1,2, Duraisamy Kempuraj2, Michael Tagen2,3, Magdalini Vasiadi4, Asimenia Angelidou5, Konstantinos-Dionysios Alyssonandros3, Dimitris Kalogeromitros4, Shahrazad Asadi6, Nikolaos Stavrianas6, Erika Peterson7, Susan Leeman8,1, and Pio Conti9

1Molecular Immunopharmacology and Drug Discovery Laboratory, Department of Pharmacology and Experimental Therapeutics, Departments of 2Biochemistry and 3Internal Medicine, Tufts University School of Medicine, and 4Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Tufts Medical Center, Boston, MA 02111; 5Allergy Clinical Research Center, Division of Allergology, Attikon General Hospital, Athens University Medical School, 12462 Athens, Greece; 62nd Department of Dermatology, Attikon General Hospital, Athens University Medical School, 12462 Athens, Greece; 7Department of Pharmacology, Boston University Medical School, Boston, MA 02118; and 8Immunology Division, Department of Oncology and Neuroscience, University of Chieti Medical School, 66100 Chieti, Italy

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The peptide substance P (SP) has been implicated in inflammatory conditions, such as psoriasis, where mast cells and VEGF are increased. A relationship between SP and VEGF has not been well studied, nor has any interaction with the proinflammatory cytokines, especially IL-33. Here we report that SP (0.1–10 μM) induces gene expression and secretion of VEGF from human LAD2 mast cells and human umbilical cord blood-derived cultured mast cells (hCBMCs). This effect is significantly increased by coadministration of IL-33 (5–100 ng/mL) in both cell types. The effect of SP on VEGF release is inhibited by treatment with the NK-1 receptor antagonist 733,060. SP rapidly increases cytosolic calcium, and so does IL-33 to a smaller extent; the addition of IL-33 augments the calcium increase. SP-induced VEGF production involves calcium-dependent PKC isoforms, as well as the ERK and JNK MAPKs. Gene expression of IL-33 and histidine decarboxylase (HDC), an indicator of mast cell presence/activation, is significantly increased in affected and unaffected (at least 15 cm away from the lesion) psoriatic skin, as compared with normal control skin. Immunohistochemistry indicates that IL-33 is associated with endothelial cells in both the unaffected and affected sites, but is stronger and also associated with immune cells in the affected site. These results imply that functional interactions among SP, IL-33, and mast cells leading to VEGF release contribute to inflammatory conditions, such as the psoriasis, an allergenic hyperproliferative skin inflammatory disorder with a neurogenic component.

inflammation | cytokines | IL-1 | innate immunity | stress

Substance P (SP) is an 11–amino acid peptide that mediates inflammation (1, 2), partially through mast cell activation (3, 4). Neuropeptides (5), especially SP, could be involved in the pathogenesis of inflammatory skin disorders, such as psoriasis (6, 7), characterized by increased epidermal vascularization, keratinocyte hyperproliferation, and inflammation (8). SP-positive nerve fibers are more dense in psoriatic lesions and have an increased number of mast cell contacts compared with normal skin (9–12). Mast cells are also increased in lesional psoriatic skin (13) and there appears to be an association among sensory nerves, mast cell numbers, and stress (13, 14). SP-positive nerve fibers and mast cell contacts are also increased by acute stress in mice, leading to dermal mast cell degranulation (3, 15, 16). It also is interesting that psoriasis is worsened by acute stress (15, 17).

Psoriatic plaques contain increased levels of VEGF compared with normal skin (18–20). VEGF is a major proangiogenic factor involved in many inflammatory diseases (21). The VEGF 121 isoform is particularly increased in psoriatic plaques (22) and VEGF is also increased systematically in severe psoriasis (22, 23). Genetic studies have shown that several different VEGF polymorphisms are associated with an increased risk of developing psoriasis (24, 25). Mast cells can secrete VEGF in response to IgE (26, 27), and to corticotropin-releasing hormone (CRH) (28), secreted under stress. Epidermal overexpression of VEGF in transgenic mice leads to a phenotype nearly identical to that of psoriasis (29).

Given that psoriasis involves skin inflammation and is often present with arthritis (psoriatic arthritis) (30), we were intrigued by the finding that IL-33 exacerbates antigen-induced arthritis in mice by activating mast cells (31). IL-33 is one of the newest members of the IL-1 family of inflammatory cytokines (32), and was recently shown to mediate IgE-induced anaphylaxis in mice (33). IL-33 also induces release of IL-6 from mouse bone marrow–derived cultured mast cells (BMCMCs) (34), and IL-8 from human umbilical cord blood–derived cultured mast cells (hCBMCs) (35).

Mast cells are found in large numbers around blood vessels in the skin, where they participate in allergic and inflammatory reactions through release of multiple mediators with potent vasodilatory, inflammatory, and nociceptive properties (36, 37). For example, CRH increases vascular permeability through release of histamine (38), which also stimulates cutaneous sensory nerves (39), contributing to pruritus. Skin mast cells may have important functions as “sensors” of environmental and emotional stress (40).

In the present study, we show that SP stimulates human mast cells to secrete VEGF and that this action is augmented by IL-33. Furthermore, we show that IL-33 mRNA expression is increased along with histidine decarboxylase (HDC), an indicator of mast cell presence/activation, in psoriatic skin.

Results

SP Stimulates VEGF mRNA Expression and Protein Production in Human Mast Cells. To examine the effect of SP on VEGF secretion, LAD2 cells were treated with SP (0.01–10 μM) for 24 h.


The authors declare no conflict of interest.

1To whom correspondence may be addressed. E-mail: theoharis.theocharides@tufts.edu or sleeman@bu.edu.

2B.Z., D.K., and M.T. contributed equally to this work.

3Present address: Department of Pharmaceutical Sciences, St. Jude Children’s Research Hospital, Memphis, TN 38105.

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Stimulation with SP caused a concentration-dependent production and release of VEGF with a maximum of 546 pg/10^6 cells at 10 μM, a 2.7-fold induction (Fig. 1A). To investigate whether this effect was limited to the leukemic human LAD2 mast cells, we repeated the experiments using hCBMCs (Fig. 1B). SP stimulation caused a maximum release from hCBMCs of 445 pg/10^6 cells at 10 μM. VEGF release in response to 1 μM SP was time dependent over 24 h (Fig. 1C). This slow VEGF secretion suggests de novo synthesis. We next examined whether the VEGF mRNA transcript is induced by SP using quantitative PCR. VEGF mRNA was increased after SP (1 μM) stimulation with a maximum ≈12-fold increase occurring at 6 h (Fig. 1D). Elevation of VEGF mRNA was sustained for at least 24 h. Together, these data show that SP induces both VEGF mRNA and protein synthesis in human mast cells.

**IL-33 Augments Effect of SP on VEGF Release from Human Mast Cells.** We next examined whether IL-33 could induce VEGF release from LAD2 mast cells. IL-33 alone (5–100 ng/mL) did not induce VEGF release (Fig. 2A). However, the addition of IL-33 (100 ng/mL) to SP (1 μM) augmented VEGF production by 1.5-fold as compared with that induced by SP alone (Fig. 2A). IL-33 alone (5–100 ng/mL) over 6 h increased VEGF gene expression up to 4.5-fold (Fig. 2B). The addition of IL-33 (100 ng/mL) to SP (0.1 or 1 μM) further increased VEGF mRNA expression (Fig. 2B). To ensure that these findings were not limited to the use of the LAD2 leukemic human mast cells, we repeated the experiments using hCBMCs, except that these cells require a higher amount of SP for stimulation. IL-33 (100 ng/mL) again did not induce any VEGF release on its own, but augmented (Fig. 2C) VEGF release induced by SP (5 μM).

Given that IL-33 belongs to the IL-1 cytokine family, we also tested whether IL-1 could induce VEGF release and whether it could augment the release due to SP. Although IL-33 (100 ng/mL) had no effect, IL-1 (10 ng/mL) induced significant VEGF release (Fig. 2C); the addition of IL-1 (10 ng/mL) to SP (5 μM) augmented the effect of SP (Fig. 2C, P < 0.05).

**NK-1 Receptor Antagonist Inhibits SP-Induced VEGF Release.** To determine whether SP-induced VEGF release is mediated through the NK-1 receptor, we preincubated LAD2 cells with the NK-1 receptor antagonist L-733,060 (10 μM) for 30 min and then during stimulation with the SP (1 μM). Treatment of LAD2 cells with L-733,060 (10 μM) completely blocked SP-induced VEGF release (Fig. 3). In fact, this antagonist also significantly reduced basal VEGF secretion (Fig. 3).

**IL-33 Augments Cytosolic Calcium Ion Levels Increased by SP.** To examine the possible mechanism of action through which IL-33 enhances the ability of SP to increase VEGF release, we measured...
their effects on intracellular calcium ion levels. SP (1 μM) significantly increased cytosolic calcium, whereas IL-33 (100 ng/mL) produced a similar but smaller increase (Fig. 4). The addition of IL-33 to SP augmented the cytosolic calcium increase due to SP (Fig. 4).

PKC Isoforms Are Involved in SP-Induced VEGF Release. We investigated whether PKC plays a role in VEGF release from LAD2 cells using two PKC inhibitors: bisindolylmaleimide I, a non-selective inhibitor of PKC, and Gö6976, an inhibitor selective for the calcium-dependent PKCα and β isoforms. Both bisindolylmaleimide I (Fig. S1A) and Gö6976 (Fig. S1B) decreased (~30%) SP-induced VEGF secretion at 1 μM without affecting basal release. These data show that the calcium/PKC pathway is important but not mandatory for maximum induction of VEGF by SP.

ERK and JNK Pathways Are Involved in SP-Induced VEGF Release. We then examined MAPK activation by performing Western blots of the active form of the MAPKs using phospho-specific antibodies. Upon stimulation with SP (1 μM), increased ERK phosphorylation was detected within 5 min, and sustained levels of phospho-ERK lasted for at least 120 min (Fig. S2A). Increased phospho-JNK was detected, but with kinetics different from those of phospho-ERK (Fig. S2B); JNK phosphorylation was more delayed, with peak activation at 45 min. SP did not cause any discernible changes in the level of p38 phosphorylation (Fig. S1C).

We also used inhibitors of the ERK, JNK, and p38 pathways to investigate their role in SP-induced VEGF release from LAD2 cells. MEK is the upstream kinase responsible for ERK phosphorylation. The MEK inhibitor PD98059 (1–50 μM) attenuated SP-induced VEGF release in a concentration-dependent fashion (Fig. S3A). PD98059 (50 μM) also significantly inhibited basal VEGF release, implicating the ERK pathway in both basal and SP-stimulated VEGF induction. SP-induced VEGF release was also inhibited in a similar manner by the JNK inhibitor SP600125 (Fig. S3B). Together, these data demonstrate that SP induces phosphorylation of both ERK and JNK in mast cells and that activation of both is necessary for maximum VEGF production.

IL-33 and HDC mRNA Expression Is Increased in Psoriatic Skin. IL-33 and HDC, an indicator of mast cell activation, mRNA expression was studied in psoriatic affected skin and unaffected skin obtained from at least 15 cm away from the lesion, as well as normal skin from healthy controls.

Analysis of skin biopsy samples from psoriatic lesional, unaffected, and control normal skin revealed that IL-33 gene expression is significantly higher in lesional psoriatic skin than in normal control skin (Fig. 5A). Expression of HDC mRNA, investigated as an index of mast cell presence/activation, was also significantly increased in lesional psoriatic skin (Fig. 5B). Interestingly, both IL-33 and HDC gene expression were also significantly increased in unaffected psoriatic skin as compared with control skin (Fig. 5). These results suggest that IL-33 and HDC gene expression is not associated with lymphocyte infiltration or keratinocyte proliferation in the psoriatic plaque. SP (TAC1) mRNA expression was increased in the unaffected skin but was lower in the affected skin (Fig. 5C).

IL-33 Immunohistochemistry. Investigation of IL-33 protein expression by immunohistochemistry showed that IL-33 was strongly associated with blood vessels, infiltrating inflammatory cells that had been exhausted (*P < 0.05 vs. control).
Discussion

We report here that IL-33 augments the SP-induced VEGF mRNA expression and VEGF protein secretion both from leukemic and normal human mast cells. IL-33 cannot induce VEGF secretion on its own. IL-33 is the newest inflammatory member of the IL-1 cytokine family (32), and we show here that IL-1 can also induce VEGF secretion from mast cells as well as augment the effect of SP. IL-1 had previously been shown to induce VEGF secretion from inflammatory cells (41).

Here we also show that gene expression of IL-33 is increased in both affected and unaffected psoriatic skin. Gene expression of HDC, indicating increased mast cell presence/activation, is also increased in both affected and unaffected psoriatic skin, as reported previously (42). Moreover, IL-33 in unaffected skin is weakly associated with blood vessels, whereas it is localized strongly with blood vessels and infiltrating inflammatory cells in the lesional affected skin. IL-33 had previously been reported to be expressed by endothelial cells (43). These results indicate that the inflammatory process may be initiated in “unaffected” skin areas where IL-33 is initially secreted by endothelial cells and augments other nonallergic triggers, such as SP, to stimulate the mast cells. In this context, any participation of IgE is not relevant, because psoriasis is not an allergic condition, unlike atopic dermatitis, which involves allergic inflammation, and where IL-33 expression was recently reported to be increased in lesional areas (33).

The receptor for IL-33 is mostly expressed on mast cells and TH2 cells, for which it acts as a chemoattractant and trigger (44). It was recently shown that IL-33-mediated mouse anaphylaxis occurred only in the presence of IgE (33). In contrast, IL-33 induced release of proinflammatory cytokines from murine mast cells (45), especially IL-6 without degranulation from BMMCDCs (34). It also enhanced IL-8 production from hCBMCs by IgE/anti-IgE stimulation, but without histamine release (35). IL-33 was also shown to augment the effect of IgE and stem cell factor (SCF) on activating mast cells and basophils (44).

The nonpeptide NK-1 receptor antagonist L-733,060 (46) blocked VEGF secretion from LAD2 cells by 100%, and also reduced basal VEGF release implying some autocrine activation. LAD2 mast cells (47) and skin mast cells (46) had previously been reported to express NK-1 receptors. The NK-1 receptor is also expressed on rat basophilic leukemia cells (47), activation of which by neurites occurred via SP (48). In contrast, murine bone marrow–derived mast cells did not release histamine in response to SP, but they did produce prostaglandin D2 and leukotrience C4 (49). Degranulation, as compared with de novo synthesis of selected mediators, may involve direct activation of G proteins (50, 51), as shown for SP (52) and the bee venom peptide mastoparan (53, 54). NK-1 receptor–independent activation of mast cells may involve activation of the MrgX2 receptor (55).

SP induces rapid cytosolic calcium increase in LAD2 cells; the addition of IL-33 further increases these levels, but to a lesser extent than what was recently reported for IL-33 addition to IgE-sensitized murine mast cells (33). Nevertheless, this augmentation of cytosolic calcium ion levels may be sufficient to lead to synergistic VEGF release. IL-33 may also induce downstream signaling steps, such as p38 activation, which was not apparent in our studies. For instance, IL-1 (from the same cytokine family as IL-33) increased p38 activation and VEGF release from human vascular smooth muscle cells (56). Moreover, SP induced p38 phosphorylation independent of ERK and JNK associated with IL-6 release from human dental pulp fibroblasts (57). Increased calcium increase in human skin mast cells subsequently activates calcium PKC isofoms (58). In this report, PKC is involved, but is not mandatory for VEGF induction. We also show that SP stimulates phosphorylation of both ERK and JNK MAP kinases, which can be activated by PKC-dependent and PKC-independent mechanisms (59, 60). Activation of these MAP kinases leads to activation of the AP-1 transcription factor, a heterodimer of c-Fos and c-Jun (61–63). The VEGF promoter has several AP-1 binding sites that increase transcription (64), a possible explanation for the increased VEGF mRNA abundance in SP-stimulated cells. Induction of VEGF by hyperbaric oxygen in human umbilical vein endothelial cells also depended on AP-1 activation by ERK and JNK (65).

Mast cells are often located close to SP-positive neuronal processes (66–69). Mast cell–neuronal interactions might be involved in the pathophysiology of psoriasis and might participate in the exacerbation of symptoms by stress (7, 13, 70). The fact that SP mRNA is increased in the unaffected skin but not in the affected areas suggests that SP is synthesized in unaffected areas and secreted from nerve terminals in the affected site (Fig. S4). Increased HDC mRNA expression in the unaffected area indicates increased mast cell presence. These mast cells in the unaffected skin may be activated by IL-33 released from endothelial and epithelial cells (43), acting together with IgE (44). Activated mast cells would then release histamine or interleukins that could activate neurons to synthesize more SP (Fig. S4). In the affected psoriatic skin, other possible sources of IL-33 may include inflamed...
trating lymphocytes, proliferating keratinocytes, as well as endo-
thelial cells from new vessels (Fig. S4). IL-33 would augment the
effect of SP on mast cells to release VEGF, thus increasing vas-
cular permeability and contributing to inflammation.

SP-positive nerve fibers were shown to be denser in psoriatic
skin (9–11) and to have increased numbers of mast cell contacts
compared with normal skin (12, 67). Use of biotinylated SP
suggests that NK-1 receptor may be increased in keratinocytes
from psoriatic plaques (71). Another study showed mast cells
express the NK-1 receptor in both affected and unaffected
psoriatic skin (6). NK-1 is also important in stress-induced
murine skin mast cell activation (3, 16), and in the development
of atopic dermatitis in mice (68). In fact, stress increases SP-
positive nerve fibers and mast cell contacts in mice (69), whereas
an NK-1 receptor antagonist inhibits stress-induced mast cell
degranulation in mice (72). SP also induces mast cell-dependent
leukocyte infiltration, thus amplifying the initial inflammatory
response (73). SP may contribute to the pruritus associated with
psoriasis (74). However, the effect of SP is apparently localized
to the skin, as plasma SP levels did not differ between psoriasis
patients and controls (75).

The ability of IL-33 to augment the effect of SP on inducing
mast cell release of VEGF is certainly relevant, as angiogenesis
(21) is at the core of psoriasis pathogenesis (18). VEGF levels
are increased in psoriatic plaques compared with normal skin
(19, 20), especially the VEGF 121 isoform, which causes vascular
permeability (22, 23). Moreover, the higher VEGF expression
leads to selective secretion of vascular endothelial growth factor.

The present results indicate that interactions among SP, IL-33,
and mast cells may be important in inflammatory diseases where
there is excessive angiogenesis, such as psoriasis. SP, IL-33 and
mast cells may also represent novel therapeutic targets.

Materials and Methods

Culture of Human Mast Cells. LAD2 cells (kindly supplied by Dr. A.S. Kirchenbaum, National Institutes of Health) derived from a human mast cell leukemia (78) were cultured in StemPro-34 medium (Invitrogen) supple-
mented with 100 U/mL penicillin/streptomycin and 100 ng/mL recombinant human stem cell factor (rhSCF; kindly supplied by Amgen). Human umbilical cord blood was collected at Tufts Medical Center. Hematopoietic stem cells (CD34+) were isolated by positive selection of CD34+AC133+ cells by magnetic cell sorting using an AC133+ cell isolation kit (Miltenyi Biotec) as previously reported (79).

Cytosolic Calcium Measurements. Detailed methods are provided in SI Materials and Methods.

Patients and Biopsies. Methods are described in SI Materials and Methods.

IL-33 Immunohistochemistry. Methods are detailed in SI Materials and Methods.

Statistical Analysis. Data are expressed as the mean ± SD. Statistical significance between experimental samples and controls was calculated using the Student's t test. P values less than 0.05 were considered statistically significant.

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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 × 10⁶ cells/well) 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.

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’-TGTGATGGTGGGAATGGGTCA-3’ and β-actin reverse, 5’-TTGTATGTTCACGCCAGATTCC-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 GAPDH (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 (10⁶ 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.

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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) Gö6976 (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).

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.

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. 54. 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.