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

Theoharis C. Theoharides[a,b,c,d,1], Bodi Zhang[a,b,2], Duraisamy Kempuraj[a,2], Michael Tagen[a,2,3], Magdalini Vasiadi[a,d], Asimenia Angelidou[a], Konstantinos-Dionysios Alysandratos[a], Dimitris Kalogeromitros[d], Shahrzad Asadi[a], Nikolaos Stavrianeas[e], Erika Peterson[f], Susan Leeman[g,1], and Pio Conti[h]

[a]Molecular Immunopharmacology and Drug Discovery Laboratory, Department of Pharmacology and Experimental Therapeutics, Departments of [b]Biochemistry and [c]Internal Medicine, Tufts University School of Medicine, and [f]Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Tufts Medical Center, Boston, MA 02111; [d]Allergy Clinical Research Center, Division of Allergology, Attikon General Hospital, Athens University Medical School, 12462 Athens, Greece; [e]2nd Department of Dermatology, Attikon General Hospital, Athens University Medical School, 12462 Athens, Greece; [g]Department of Pharmacology, Boston University Medical School, Boston, MA 02118; and [h]Immunology Division, Department of Oncology and Neuroscience, University of Chieti Medical School, 66100 Chieti, Italy

Contributed by Susan E. Leeman, January 22, 2010 (sent for review October 30, 2009)

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 core 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, a nonallergic 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.

Author contributions: T.C.T., B.Z., D. Kempuraj, M.T., A.A., K.-D.A., and P.C. designed research; B.Z., D. Kempuraj, M.T., M.V., A.A., K.-D.A., and S.A. performed research; D. Kalogeromitros, N.S., and E.P. contributed new reagents/analytic tools; T.C.T., B.Z., D. Kempuraj, M.T., M.V., A.A., K.-D.A., S.L., and P.C. analyzed data; and T.C.T., B.Z., D. Kempuraj, M.T., and S.E.L. wrote the paper.

The authors declare no conflict of interest.

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

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

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

This article contains supporting information online at www.pnas.org/cgi/content/full/1000803107/DCSupplemental.

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. 1*A*). To investigate whether this effect was limited to the leukemic human LAD2 mast cells, we repeated the experiments using hCBMCs (Fig. 1*B*). 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. 1*C*). 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. 1*D*). 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. 2*A*). 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. 2*A*). IL-33 alone (5–100 ng/mL) over 6 h increased VEGF gene expression up to 4.5-fold (Fig. 2*B*). The addition of IL-33 (100 ng/mL) to SP (0.1 or 1 μM) further increased VEGF mRNA expression (Fig. 2*B*). To ensure that these findings were not limited to the use of the LAD2 leukemic human mast cells, we repeated the experiments unsing 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. 2*C*) 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. 2*C*); the addition of IL-1 (10 ng/mL) to SP (5 μM) augmented the effect of SP (Fig. 2*C*, $P < 0.05$).

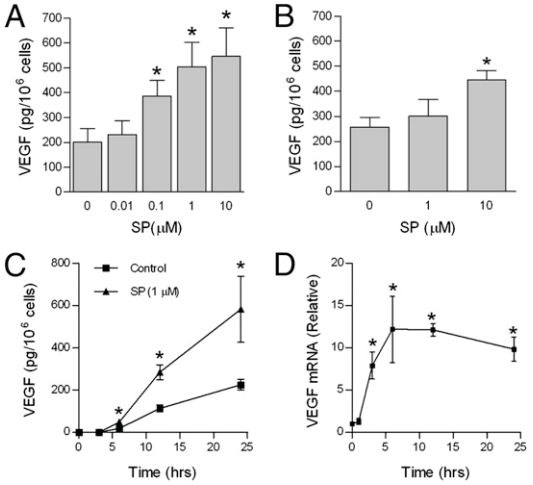

Fig. 1. SP stimulates VEGF production in human mast cells. LAD2 cells (*A*) and hCBMCs (*B*) were stimulated with the indicated concentration of SP (0–10 μM) for 24 h, and supernatant VEGF was measured by ELISA. (*C*) Time-dependent secretion of VEGF. Cells were stimulated with SP (1 μM) or vehicle for the indicated times and supernatant VEGF was measured by ELISA. (*D*) SP induces VEGF mRNA. LAD2 cells were stimulated with SP (1 μM) for the indicated times, RNA was extracted, and relative VEGF mRNA levels were determined by real-time PCR. Data are mean ± SD of three separate experiments performed in triplicate (*$P < 0.05$ vs. unstimulated cells).

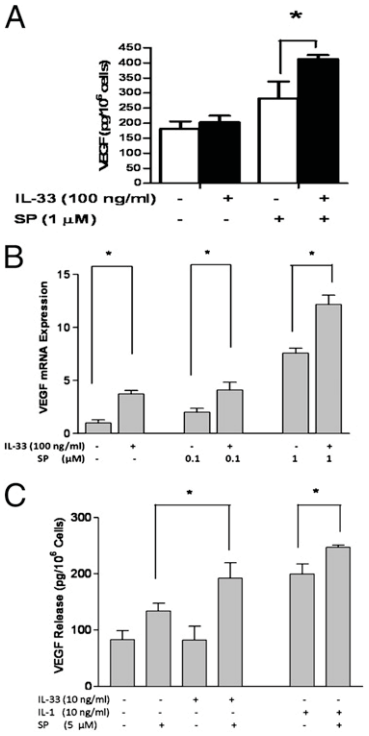

Fig. 2. IL-33 augments SP in inducing (*A*) VEGF protein secretion from LAD2 cells, and (*B*) VEGF mRNA expression from LAD2 cells, or (*C*) VEGF protein secretion from hCBMCs. Cells were treated for 6 h with IL-33 (100 ng/mL) or IL-1 (10 ng/mL) alone or together with SP as shown ($n = 3$). *$P < 0.05$.

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

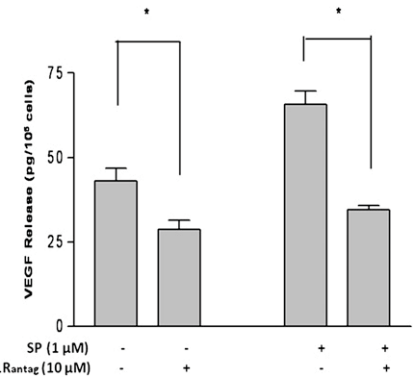

Fig. 3. NK-1 receptor antagonist inhibits SP-induced VEGF release from LAD2 cells. LAD2 cells were pretreated with NK-1 receptor antagonist (NK1RAntag) L-733,060 (10 μM) for 30 min and were then retained throughout stimulation with SP (1 μM) for 24 h. VEGF was measured in the supernatant fluid by ELISA ($n = 3$). *$P < 0.05$.

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 nonselective inhibitor of PKC, and Gö6976, an inhibitor selective for the calcium-dependent PKCα and βI isoforms. Both bisindolylmaleimide I (Fig. S1*A*) and Gö6976 (Fig. S1*B*) 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. S2*A*). Increased phospho-JNK was detected, but with kinetics different from those of phospho-ERK (Fig. S2*B*); 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. S1*C*).

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. S3*A*). 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. S3*B*). 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.

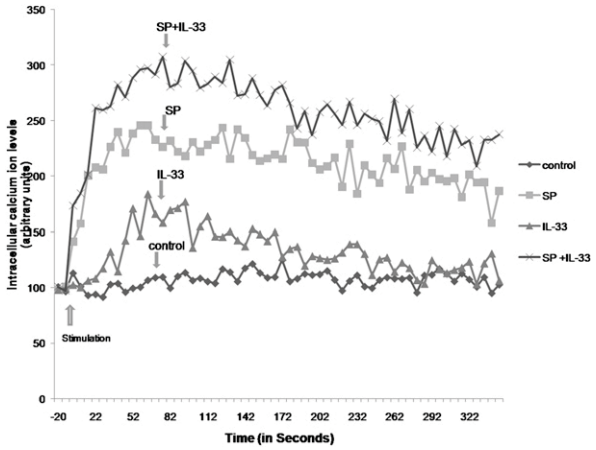

Fig. 4. Effect of SP and IL-33 on LAD2 cytosolic calcium levels. Cytosolic calcium was measured in LAD2 cells using Fura-2 AM (1 mM; Invitrogen). Cells were stimulated with IL-33 (100 ng/mL) or SP (1 μM) or both for the time indicated. Results were processed according to the Invitrogen Fura-2 protocol. Stimulation was carried out in the presence of extracellular calcium (1 mM). Results of one experiment, representative of three equivalent experiments, are shown.

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. 5*A*). Expression of HDC mRNA, investigated as an index of mast cell presence/activation, was also significantly increased in lesional psoriatic skin (Fig. 5*B*). 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. 5*C*).

IL-33 Immunohistochemistry. Investigation of IL-33 protein expression by immunohistochemistry showed that IL-33 was strongly associated with blood vessels, infiltrating inflammatory

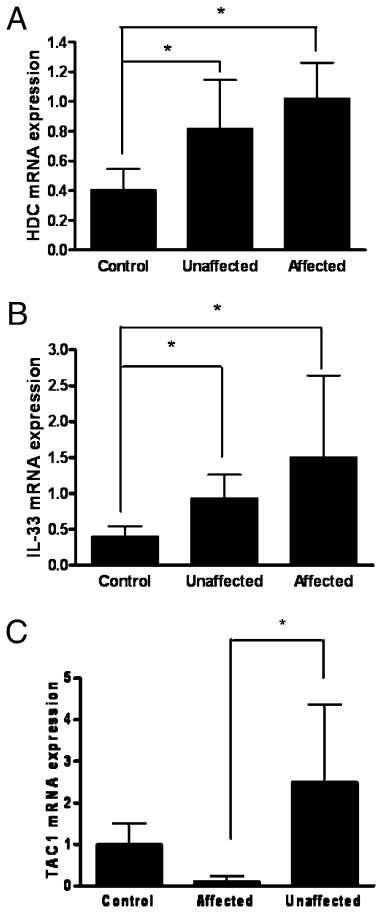

Fig. 5. Increased gene expression in psoriatic affected (lesional) skin, psoriatic unaffected (at least 15 cm away from the lesion) skin, and normal skin from healthy controls. (*A*) IL-33 (control $n = 7$; unaffected, $n = 6$; affected $n = 9$); (*B*) HDC (control $n = 5$; unaffected $n = 6$; affected $n = 5$); and (*C*) TACI. Relative quantities of mRNA expression were measured by quantitative RT-PCR and normalized to 18S. TaqMan was performed with cDNA reverse transcribed from 100 ng RNA from each sample. The number of samples was less for HDC because the amount of cDNA from some samples had been exhausted (*$P < 0.05$ vs. control).

cells, and sweat glands in the affected psoriatic skin areas (Fig. 6*A*). On the contrary, IL-33 was weakly associated with blood vessels and sweat glands in unaffected psoriatic areas (Fig. 6*B*). There was no apparent association with mast cells.

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).

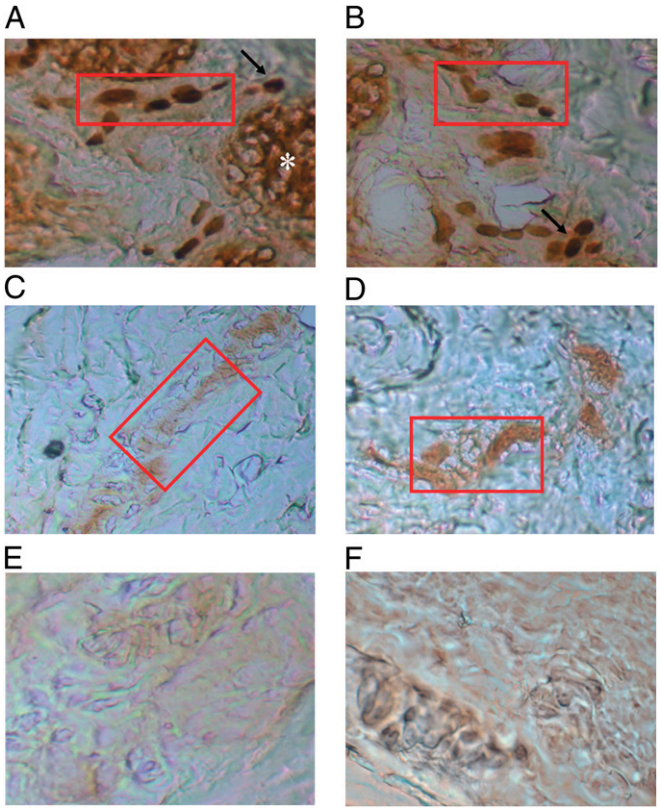

Fig. 6. Photomicrographs of skin biopsy samples from patients with psoriasis (*A* and *B*) lesional, affected skin; (*C* and *D*) unaffected skin; and (*E* and *F*) control without primary antibody. Immunohistochemical staining was performed using the LSAB+ system kit (DAKO). Incubation with the primary 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. Magnification, ×200; Red rectangle indicates blood vessel; asterisk indicates sweat gland; solid arrow indicates inflammatory cells.

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 BMCMCs (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 D_2 and leukotrience C_4 (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).

SP-induced calcium increase in human skin mast cells subsequently activates calcium PKC isoforms (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 infil-

trating lymphocytes, proliferating keratinocytes, as well as endothelial cells from new vessels (Fig. S4). IL-33 would augment the effect of SP on mast cells to release VEGF, thus increasing vascular 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 suggested that NK-1 expression 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 correlates with the clinical severity of psoriasis (76, 77). Genetic studies have shown that several different VEGF polymorphisms are associated with an increased risk of developing psoriasis (24, 25). Moreover, transgenic delivery of VEGF in mouse skin can lead to an inflammatory state resembling psoriasis (29).

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. Kirshenbaum, National Institutes of Health) derived from a human mast cell leukemia (78) were cultured in StemPro-34 medium (Invitrogen) supplemented 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 (Milltenyi 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.

ACKNOWLEDGMENTS. We thank Amgen, Inc. (Thousand Oaks, CA) for the kind gift of rhSCF and Drs. Dean Metcalfe and A.S. Kirshenbaum, National Insitutes of Health (NIH) for the LAD2 mast cells. We also thank Jessica Christian for word processing skills. This work was supported in part by NIH Grant R01 AR47652 (to T.C.T.). Both K.-D.A. and A.A. are recipients of postgraduate scholarships from the Hellenic State Scholarships Foundation (Athens, Greece).

1. Leeman SE, Ferguson SL (2000) Substance P: An historical perspective. *Neuropeptides* 34:249–254.
2. O'Connor TM, et al. (2004) The role of substance P in inflammatory disease. *J Cell Physiol* 201:167–180.
3. Kawana S, Liang Z, Nagano M, Suzuki H (2006) Role of substance P in stress-derived degranulation of dermal mast cells in mice. *J Dermatol Sci* 42:47–54.
4. Kandere-Grzybowska K, et al. (2003) Stress-induced dura vascular permeability does not develop in mast cell-deficient and neurokinin-1 receptor knockout mice. *Brain Res* 980:213–220.
5. Saraceno R, Kleyn CE, Terenghi G, Griffiths CE (2006) The role of neuropeptides in psoriasis. *Br J Dermatol* 155:876–882.
6. Remröd C, Lonne-Rahm S, Nordlind K (2007) Study of substance P and its receptor neurokinin-1 in psoriasis and their relation to chronic stress and pruritus. *Arch Dermatol Res* 299:85–91.
7. Farber EM, Nickoloff BJ, Recht B, Fraki JE (1986) Stress, symmetry, and psoriasis: Possible role of neuropeptides. *J Am Acad Dermatol* 14:305–311.
8. Schön MP, Boehncke WH (2005) Psoriasis. *N Engl J Med* 352:1899–1912.
9. Jiang WY, Raychaudhuri SP, Farber EM (1998) Double-labeled immunofluorescence study of cutaneous nerves in psoriasis. *Int J Dermatol* 37:572–574.
10. Chan J, Smoller BR, Raychauduri SP, Jiang WY, Farber EM (1997) Intraepidermal nerve fiber expression of calcitonin gene-related peptide, vasoactive intestinal peptide and substance P in psoriasis. *Arch Dermatol Res* 289:611–616.
11. Al'Abadie MS, Senior HJ, Bleehen SS, Gawkrodger DJ (1995) Neuropeptides and general neuronal marker in psoriasis—an immunohistochemical study. *Clin Exp Dermatol* 20:384–389.
12. Naukkarinen A, et al. (1996) Quantitative histochemical analysis of mast cells and sensory nerves in psoriatic skin. *J Pathol* 180:200–205.
13. Özdamar SO, Seçkin D, Kandemir B, Turanli AY (1996) Mast cells in psoriasis. *Dermatology* 192:190.
14. Harvima IT, et al. (1993) Association of cutaneous mast cells and sensory nerves with psychic stress in psoriasis. *Psychother Psychosom* 60:168–176.
15. Fortune DG, Richards HL, Griffiths CE (2005) Psychologic factors in psoriasis: Consequences, mechanisms, and interventions. *Dermatol Clin* 23:681–694.
16. Paus R, Heinzelmann T, Robicsek S, Czarnetzki BM, Maurer M (1995) Substance P stimulates murine epidermal keratinocyte proliferation and dermal mast cell degranulation in situ. *Arch Dermatol Res* 287:500–502.
17. Harvima RJ, et al. (1996) Association of psychic stress with clinical severity and symptoms of psoriatic patients. *Acta Derm Venereol* 76:467–471.
18. Heidenreich R, Röcken M, Ghoreschi K (2009) Angiogenesis drives psoriasis pathogenesis. *Int J Exp Pathol* 90:232–248.
19. Yalçin B, Tezel GG, Arda N, Erman M, Alli N (2007) Vascular endothelial growth factor, vascular endothelial growth factor receptor-3 and cyclooxygenase-2 expression in psoriasis. *Anal Quant Cytol Histol* 29:358–364.
20. Simonetti O, et al. (2006) VEGF is likely a key factor in the link between inflammation and angiogenesis in psoriasis: Results of an immunohistochemical study. *Int J Immunopathol Pharmacol* 19:751–760.
21. Byrne AM, Bouchier-Hayes DJ, Harmey JH (2005) Angiogenic and cell survival functions of vascular endothelial growth factor (VEGF). *J Cell Mol Med* 9:777–794.
22. Creamer D, et al. (2002) Mediation of systemic vascular hyperpermeability in severe psoriasis by circulating vascular endothelial growth factor. *Arch Dermatol* 138: 791–796.
23. Zhang Y, Matsuo H, Morita E (2005) Vascular endothelial growth factor 121 is the predominant isoform in psoriatic scales. *Exp Dermatol* 14:758–764.
24. Detmar M (2004) Evidence for vascular endothelial growth factor (VEGF) as a modifier gene in psoriasis. *J Invest Dermatol* 122:209–215.
25. Barile S, et al. (2006) Vascular endothelial growth factor gene polymorphisms increase the risk to develop psoriasis. *Exp Dermatol* 15:368–376.
26. Boesiger J, et al. (1998) Mast cells can secrete vascular permeability factor/vascular endothelial cell growth factor and exhibit enhanced release after immunoglobulin E-dependent upregulation of fc ε receptor I expression. *J Exp Med* 188:1135–1145.
27. Grützkau A, et al. (1998) Synthesis, storage, and release of vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) by human mast cells: Implications for the biological significance of $VEGF_{206}$. *Mol Biol Cell* 9:875–884.
28. Cao J, et al. (2005) Human mast cells express corticotropin-releasing hormone (CRH) receptors and CRH leads to selective secretion of vascular endothelial growth factor. *J Immunol* 174:7665–7675.
29. Xia YP, et al. (2003) Transgenic delivery of VEGF to mouse skin leads to an inflammatory condition resembling human psoriasis. *Blood* 102:161–168.
30. Nestle FO, Kaplan DH, Barker J (2009) Psoriasis. *N Engl J Med* 361:496–509.
31. Xu D, et al. (2008) IL-33 exacerbates antigen-induced arthritis by activating mast cells. *Proc Natl Acad Sci USA* 105:10913–10918.
32. Castellani ML, et al. (2009) The latest interleukin: IL-33 the novel IL-1-family member is a potent mast cell activator. *J Biol Regul Homeost Agents* 23:11–14.
33. Pushparaj PN, Tay HK, H'ng SC, Pitman N, Xu D, et al. (2009) The cytokine interleukin-33 mediates anaphylactic shock. *Proc Natl Acad Sci USA* 106:9773–9778.
34. Ho LH, et al. (2007) IL-33 induces IL-13 production by mouse mast cells independently of IgE-FcepsilonRI signals. *J Leukoc Biol* 82:1481–1490.
35. Iikura M, et al. (2007) IL-33 can promote survival, adhesion and cytokine production in human mast cells. *Lab Invest* 87:971–978.
36. Theoharides TC, Cochrane DE (2004) Critical role of mast cells in inflammatory diseases and the effect of acute stress. *J Neuroimmunol* 146:1–12.
37. Castells M (2006) Mast cell mediators in allergic inflammation and mastocytosis. *Immunol Allergy Clin North Am* 26:465–485.
38. Theoharides TC, et al. (1998) Corticotropin-releasing hormone induces skin mast cell degranulation and increased vascular permeability, a possible explanation for its proinflammatory effects. *Endocrinology* 139:403–413.

39. Christian EP, Undem BJ, Weinreich D (1989) Endogenous histamine excites neurones in the guinea-pig superior cervical ganglion *in vitro*. *J Physiol* 409:297–312.
40. Paus R, Theoharides TC, Arck PC (2006) Neuroimmunoendocrine circuitry of the 'brain-skin connection'. *Trends Immunol* 27:32–39.
41. Salven P, Hattori K, Heissig B, Rafii S (2002) Interleukin-1alpha promotes angiogenesis in vivo via VEGFR-2 pathway by inducing inflammatory cell VEGF synthesis and secretion. *FASEB J* 16:1471–1473.
42. Michaëlsson G, et al. (1997) The skin and the gut in psoriasis: The number of mast cells and CD3+ lymphocytes is increased in non-involved skin and correlated to the number of intraepithelial lymphocytes and mast cells in the duodenum. *Acta Derm Venereol* 77:343–346.
43. Moussion C, Ortega N, Girard JP (2008) The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells in vivo: A novel 'alarmin'? *PLoS One* 3:e3331.
44. Silver MR, et al. (2009) IL-33 synergizes with IgE-dependent and IgE-independent agents to promote mast cell and basophil activation. *Inflamm Res*, 10.1007/s00011-009-0088-5.
45. Moulin D, et al. (2007) Interleukin (IL)-33 induces the release of pro-inflammatory mediators by mast cells. *Cytokine* 40:216–225.
46. Seabrook GR, et al. (1996) L-733,060, a novel tachykinin NK1 receptor antagonist; effects in [Ca2+]i mobilisation, cardiovascular and dural extravasation assays. *Eur J Pharmacol* 317:129–135.
47. Cooke HJ, Fox P, Alferes L, Fox CC, Wolfe SA, Jr (1998) Presence of NK_1 receptors on a mucosal-like mast cell line, RBL-2H3 cells. *Can J Physiol Pharmacol* 76:188–193.
48. Suzuki R, et al. (1999) Direct neurite-mast cell communication *in vitro* occurs via the neuropeptide substance P. *J Immunol* 163:2410–2415.
49. Karimi K, Kool M, Nijkamp FP, Redegeld FA (2004) Substance P can stimulate prostaglandin D2 and leukotriene C4 generation without granule exocytosis in murine mast cells. *Eur J Pharmacol* 489:49–54.
50. Mousli M, Bueb J-L, Bronner C, Rouot B, Landry Y (1990) G protein activation: A receptor-independent mode of action for cationic amphiphilic neuropeptides and venom peptides. *Trends Pharmacol Sci* 11:358–362.
51. Ferry X, Brehin S, Kamel R, Landry Y (2002) G protein-dependent activation of mast cell by peptides and basic secretagogues. *Peptides* 23:1507–1515.
52. Mousli M, Bronner C, Landry Y, Bockaert J, Rouot B (1990) Direct activation of GTP-binding regulatory proteins (G-proteins) by substance P and compound 48/80. *FEBS Lett* 259:260–262.
53. Mousli M, Bronner C, Bueb JL, Tschirhart E, Gies JP, Landry Y (1989) Activation of rat peritoneal mast cells by substance P and mastoparan. *J Pharmacol Exp Ther* 250(1): 329–335.
54. Higashijima T, Uzu S, Nakajima T, Ross EM (1988) Mastoparan, a peptide toxin from wasp venom, mimics receptors by activating GTP-binding regulatory proteins (G proteins). *J Biol Chem* 263:6491–6494.
55. Tatemoto K, et al. (2006) Immunoglobulin E-independent activation of mast cell is mediated by Mrg receptors. *Biochem Biophys Res Commun* 349:1322–1328.
56. Jung YD, et al. (2001) Vascular endothelial growth factor is upregulated by interleukin-1 beta in human vascular smooth muscle cells via the P38 mitogen-activated protein kinase pathway. *Angiogenesis* 4:155–162.
57. Tokuda M, Miyamoto R, Sakuta T, Nagaoka S, Torii M (2005) Substance P activates p38 mitogen-activated protein kinase to promote IL-6 induction in human dental pulp fibroblasts. *Connect Tissue Res* 46:153–158.
58. Columbo M, Botana LM, Horowitz EM, Lichtenstein LM, MacGlashan DW, Jr (1994) Studies of the intracellular Ca2+ levels in human adult skin mast cells activated by the ligand for the human c-kit receptor and anti-IgE. *Biochem Pharmacol* 47:2137–2145.
59. Zhang C, Hirasawa N, Beaven MA (1997) Antigen activation of mitogen-activated protein kinase in mast cells through protein kinase C-dependent and independent pathways. *J Immunol* 158:4968–4975.
60. Kawakami Y, Hartman SE, Holland PM, Cooper JA, Kawakami T (1998) Multiple signaling pathways for the activation of JNK in mast cells: Involvement of Bruton's tyrosine kinase, protein kinase C, and JNK kinases, SEK1 and MKK7. *J Immunol* 161: 1795–1802.
61. Dérijard B, et al. (1994) JNK1: A protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell* 76:1025–1037.
62. Kyriakis JM, et al. (1994) The stress-activated protein kinase subfamily of c-Jun kinases. *Nature* 369:156–160.
63. Frost JA, Geppert TD, Cobb MH, Feramisco JR (1994) A requirement for extracellular signal-regulated kinase (ERK) function in the activation of AP-1 by Ha-Ras, phorbol 12-myristate 13-acetate, and serum. *Proc Natl Acad Sci USA* 91:3844–3848.
64. Tischer E, et al. (1991) The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing. *J Biol Chem* 266:11947–11954.
65. Lee CC, et al. (2006) Hyperbaric oxygen induces VEGF expression through ERK, JNK and c-Jun/AP-1 activation in human umbilical vein endothelial cells. *J Biomed Sci* 13: 143–156.
66. Wiesner-Menzel L, Schulz B, Vakilzadeh F, Czarnetzki BM (1981) Electron microscopical evidence for a direct contact between nerve fibres and mast cells. *Acta Derm Venereol* 61:465–469.
67. Naukkarinen A, Harvima I, Paukkonen K, Aalto M-L, Horsmanheimo M (1993) Immunohistochemical analysis of sensory nerves and neuropeptides, and their contacts with mast cells in developing and mature psoriatic lesions. *Arch Dermatol Res* 285:341–346.
68. Pavlovic S, et al. (2008) Further exploring the brain-skin connection: Stress worsens dermatitis via substance P-dependent neurogenic inflammation in mice. *J Invest Dermatol* 128:434–446.
69. Peters EM, et al. (2005) Stress exposure modulates peptidergic innervation and degranulates mast cells in murine skin. *Brain Behav Immun* 19:252–262.
70. Al'Abadie MS, Kent GG, Gawkrodger DJ (1994) The relationship between stress and the onset and exacerbation of psoriasis and other skin conditions. *Br J Dermatol* 130: 199–203.
71. Staniek V, Doutremepuich J, Schmitt D, Claudy A, Misery L (1999) Expression of substance P receptors in normal and psoriatic skin. *Pathobiology* 67:51–54.
72. Erin N, Ersoy Y, Ercan F, Akici A, Oktay S (2004) NK-1 antagonist CP99994 inhibits stress-induced mast cell degranulation in rats. *Clin Exp Dermatol* 29:644–648.
73. Matsuda H, Kawakita K, Kiso Y, Nakano T, Kitamura Y (1989) Substance P induces granulocyte infiltration through degranulation of mast cells. *J Immunol* 142:927–931.
74. Nakamura M, Toyoda M, Morohashi M (2003) Pruritogenic mediators in psoriasis vulgaris: Comparative evaluation of itch-associated cutaneous factors. *Br J Dermatol* 149:718–730.
75. Reich A, Orda A, Wiśnicka B, Szepietowski JC (2007) Plasma concentration of selected neuropeptides in patients suffering from psoriasis. *Exp Dermatol* 16:421–428.
76. Detmar M, et al. (1994) Overexpression of vascular permeability factor/vascular endothelial growth factor and its receptors in psoriasis. *J Exp Med* 180:1141–1146.
77. Bhushan M, McLaughlin B, Weiss JB, Griffiths CE (1999) Levels of endothelial cell stimulating angiogenesis factor and vascular endothelial growth factor are elevated in psoriasis. *Br J Dermatol* 141:1054–1060.
78. Kirshenbaum AS, et al. (2003) Characterization of novel stem cell factor responsive human mast cell lines LAD 1 and 2 established from a patient with mast cell sarcoma/leukemia; activation following aggregation of FcepsilonRI or FcgammaRI. *Leuk Res* 27:677–682.
79. Kempuraj D, et al. (1999) Characterization of mast cell-committed progenitors present in human umbilical cord blood. *Blood* 93:3338–3346.