

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

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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 μ M) 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, 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 (BMCs) (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 μ M) for 24 h.

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cells, and sweat glands in the affected psoriatic skin areas (Fig. 6A). On the contrary, IL-33 was weakly associated with blood vessels and sweat glands in unaffected psoriatic areas (Fig. 6B). 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).

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 D₂ and leukotriene 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).

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-

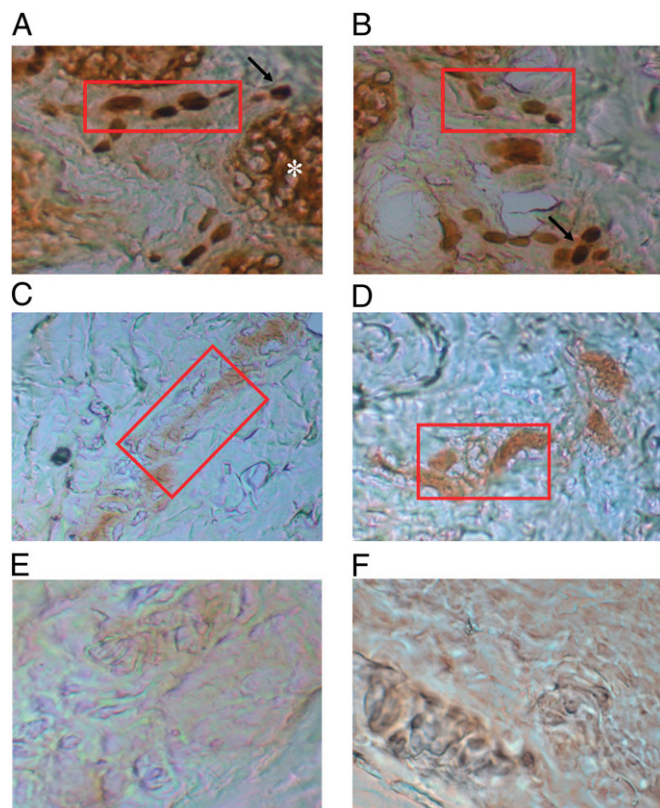


Fig. 6. Photomicrographs of skin biopsy samples from patients with psoriasis (A and B) lesional, affected skin; (C and D) unaffected skin; and (E and F) control without primary antibody. Immunohistochemical staining was performed using the LSAB+ system kit (DAKO). Incubation with the primary antibody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

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 \pm 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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